Access DB# 134888

31

SEARCH REQUEST FORM

Scientific and Technical Information Center

Requester's Full Name: Javier G. Blanco Examiner #: 703-605-4259 Date: October 12, 2004
Art Unit: 3738 Phone Number 30 Serial Number: 10/722,279
Mail Box and Bldg/Room Location: _____ Results Format Preferred (circle): PAPER DISK E-MAIL

If more than one search is submitted, please prioritize searches in order of need.

****

Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc, if known. Please attach a copy of the cover sheet, pertinent claims, and abstract.

Title of Invention: Cardiac valve replacement

Inventors (please provide full names): Richard A. Hopkins
Diane Hoffman-Kinn

Earliest Priority Filing Date: April 7, 2000

For Sequence Searches Only *Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.*

Division of 09/828,768 [now US 6,652,583]

→ Seeding isolated myofibroblasts into an acellular or decellularized matrix or graft

→ Culturing said myofibroblasts under pulsatile flow conditions

→ Said myofibroblasts produce at least two-fold more type I collagen when compared to type III collagen

****

STAFF USE ONLY	Type of Search		Vendors and cost where applicable	
Searcher: David Holloway	NA Sequence (#)		STN	
Searcher Phone #: 571-272-3328	AA Sequence (#)		Dialog	#
Searcher Location: RND 4B19	Structure (#)		Questel/Orbit	
Date Searcher Picked Up: 10-20-04	Bibliographic	4	Dr.Link	
Date Completed: 10-21-04	Litigation		Lexis/Nexis	
Searcher Prep & Review Time: 65	Fulltext	4	Sequence Systems	
Clerical Prep Time:	Patent Family		WWW/Internet	✓
Online Time: 198	Other		Other (specify)	

PTO-1590 (8-01)

STIC Search Report
EIC 2100

STIC Database Tracking Number: 134888

TO: Javier G Blanco **Location: cp2 2b08** **Art Unit : 3738** **Thursday, October 21, 2004** **Case Serial Number: 10/722279**	**From: David Holloway** **Location: EIC 2100** **RND 4B19** **Phone: 571-272-3528** **david.holloway@uspto.gov**

Search Notes

Dear Examiner Blanco,

Attached please find your search results for above-referenced case.
Please contact me if you have any questions or would like a re-focused search.

David

```
Set     Items   Description
S1      25466   MYOFIBROBLAST? OR FIBROBLAST? OR ENDOTHELIAL?
S2       1166   ACELLULAR? OR DECELLULAR? OR DE()CELLULAR?
S3     100687   MATRIX? OR LEAFLET()INTERSTITIAL()TISSUE? OR SEEDING OR FR-
             AME? OR SCAFFOLD? OR MESH? OR GRAFT? OR ALLOGRAFT? OR SEEDED -
             OR SEEDS OR SEED
S4      11258   COLLAGEN?
S5       1129   TISSUE()ENGINEER? OR BIOPROSTHET?
S6     638290   CULTUR? OR GROW? OR PRODUC? OR CLONE? OR REPLICAT? OR CLON-
             ING OR REPRODUC?
S7      93260   PULSAT? OR FLOW? OR PERFUS?
S8     202956   CARDIAC? OR VALV? OR MITRAL? OR PULMONAR? OR TRICUSPID? OR
             HEART? OR CARDIOLOG?
S9        544   S1(2N)S6 (10N) S4
S10         5   S9 (S) S3 (S) S2
S11        17   S9 (S) S7
S12        20   S9(S)(S2 OR S3)(S)(S8 OR S5)
S13        37   S10 OR S11 OR S12
S14        37   RD (unique items)
S15       731   COLLAGEN(N)TYPE? OR COLLAGEN()(ONE OR 1 OR I OR III OR 3 OR
             THREE)
S16        63   S1(3N)S6(S)S15
S17        28   S16(S)(S5 OR S7 OR S3)
S18        62   S14 OR S17
S19        62   RD (unique items)
S20        45   S19 NOT PY>2000
S21        42   S20 NOT PD=20000407:20020407
S22        42   S21 NOT PD=20020407:20041022
File  98:General Sci Abs/Full-Text 1984-2004/Aug
         (c) 2004 The HW Wilson Co.
File 135:NewsRx Weekly Reports 1995-2004/Oct W2
         (c) 2004 NewsRx
File 149:TGG Health&Wellness DB(SM) 1976-2004/Sep W4
         (c) 2004 The Gale Group
File 369:New Scientist 1994-2004/Oct W2
         (c) 2004 Reed Business Information Ltd.
File 370:Science 1996-1999/Jul W3
         (c) 1999 AAAS
File 444:New England Journal of Med. 1985-2004/Oct W2
         (c) 2004 Mass. Med. Soc.
```

22/3,K/1 **(Item 1 from file: 98)**
DIALOG(R)File 98:General Sci Abs/Full-Text

04364925 H.W. WILSON RECORD NUMBER: BGSA00114925
Growth factor ultrafiltration in experimental diabetic nephropathy contributes to interstitial fibrosis.
Wang, Shi-Nong
Hirschberg, Raimund
American Journal of Physiology (Am J Physiol) v. 278 no4 (Apr. 2000 pt2) p. F554-F560
SPECIAL FEATURES: bibl il ISSN: 0002-9513
LANGUAGE: English
COUNTRY OF PUBLICATION: United States

...ABSTRACT: the hypothesis is examined that ultrafiltered HGF and TGF-b induce increased expression of extracellular **matrix** (ECM) proteins directly in tubular cells, or induce increased expression of cytokines that may act on interstitial **myofibroblasts** . Incubation of **cultured** tubular cells with recombinant human (rh) TGF-b modestly raises expression of **collagen type** III, but rhHGF dose dependently blocks expression of this ECM protein. Both growth factors raise...

...increase expression of platelet-derived growth factor (PDGF)-BB up to sixfold, but not of **fibroblast growth** factor-2. Pooled, diluted glomerular ultrafiltrate that had been collected by nephron micropuncture from rats...

...expression. In NRK-49F renal interstitial myofibroblasts, rhPDGF-BB, in turn, raises the expression of **collagen type** III but not type I or fibronectin. The findings provide evidence for ultrafiltered HGF and...

22/3,K/6 **(Item 6 from file: 98)**
DIALOG(R)File 98:General Sci Abs/Full-Text

03522570 H.W. WILSON RECORD NUMBER: BGSI97022570

Thrombin modulates vectorial secretion of extracellular matrix proteins in cultured endothelial cells.

Papadimitriou, Evangelia
Manolopoulos, Vangelis G; Hayman, G. Thomas

American Journal of Physiology (Am J Physiol) v. 272 (Apr. '97 pt 1) p. C1112-C1122
DOCUMENT TYPE: Feature Article
SPECIAL FEATURES: bibl il ISSN: 0002-9513
LANGUAGE: English
COUNTRY OF PUBLICATION: United States

ABSTRACT: We have identified a novel cellular action of thrombin on **cultured** rat adrenal medullary **endothelial** cells (RAMEC). Five-minute incubation of RAMEC with physiological concentrations of thrombin (<1 U/ml) caused within 3 h an increase in the basolateral deposition of the extracellular **matrix** (ECM) proteins fibronectin, laminin, and collagens IV and I, concomitant with a corresponding decrease in...

...synthesis. Maximal amounts of deposited proteins increased between 2.5-fold (fibronectin) and 4-fold (**collagen** **I**) over baseline values. Similar results were obtained with thrombin receptor agonist peptide (TRAP), proteolytically active...

22/3,K/7 **(Item 7 from file: 98)**
DIALOG(R)File 98:General Sci Abs/Full-Text

03262544 H.W. WILSON RECORD NUMBER: BGSI96012544
Collagen and collagenase gene expression in three-dimensional collagen lattices are differentially regulated by a1b1 and a2b1 integrins.
Langholz, Oliver
Rockel, Dagmar; Mauch, Cornelia
The Journal of Cell Biology (J Cell Biol) v. 131 no6 (Dec. '95 pt2) p. 1903-15
DOCUMENT TYPE: Feature Article
SPECIAL FEATURES: bibl il ISSN: 0021-9525
LANGUAGE: English
COUNTRY OF PUBLICATION: United States

ABSTRACT: The regulation of collagen metabolism during extracellular **matrix** remodeling in contracting collagen lattices was examined. The study indicates that a1b1 integrin is the receptor that controls the down-regulation of **collagen I** synthesis in **fibroblasts grown** within contracting collagen lattices. a2b1 integrin appears to control the induction of **matrix** metalloproteinase-1 in this system. In addition, protein phosphorylation plays a role in the further...

```
Set     Items   Description
S1    1156712   MYOFIBROBLAST? OR FIBROBLAST? OR ENDOTHELIAL?
S2      14926   ACELLULAR? OR DECELLULAR? OR DE()CELLULAR?
S3    4446946   MATRIX? OR LEAFLET()INTERSTITIAL()TISSUE? OR SEEDING OR FR-
            AME? OR SCAFFOLD? OR MESH? OR GRAFT? OR ALLOGRAFT? OR SEEDED -
            OR SEEDS OR SEED
S4     507787   COLLAGEN?
S5      26143   TISSUE()ENGINEER? OR BIOPROSTHET?
S6   17316763   CULTUR? OR GROW? OR PRODUC? OR CLONE? OR REPLICAT? OR CLON-
            ING OR REPRODUC?
S7    3326745   PULSAT? OR FLOW? OR PERFUS?
S8    4289136   CARDIAC? OR VALV? OR MITRAL? OR PULMONAR? OR TRICUSPID? OR
            HEART? OR CARDIOLOG?
S9        263   S1(2N)S6(5N)S4(S)S7
S10        14   S1 AND S2 AND S3 AND S4 AND S5 AND S6 AND S7
S11        37   S9 AND (COLLAGEN()TYPE? OR COLLAGEN()(1 OR I OR ONE OR THR-
            EE OR 3 OR III))
S12        10   S1 AND S2 AND S4 AND S5 AND S6 AND S7 AND S8
S13        24   S1(2N)S6 AND S2 AND S3 AND S4 AND S5
S14        74   S10 OR S11 OR S12 OR S13
S15        38   RD (unique items)
S16        18   S15 NOT PY>2000
S17        15   S16 NOT PD=20000407:20020407
S18        15   S17 NOT PD=20020407:20041022
File   5:Biosis Previews(R) 1969-2004/Oct W3
         (c) 2004 BIOSIS
File  34:SciSearch(R) Cited Ref Sci 1990-2004/Oct W3
         (c) 2004 Inst for Sci Info
File  35:Dissertation Abs Online 1861-2004/Sep
         (c) 2004 ProQuest Info&Learning
File  65:Inside Conferences 1993-2004/Oct W3
         (c) 2004 BLDSC all rts. reserv.
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         (c) 2004 Elsevier Science B.V.
File  94:JICST-EPlus 1985-2004/Sep W3
         (c)2004 Japan Science and Tech Corp(JST)
File  71:ELSEVIER BIOBASE 1994-2004/Oct W2
         (c) 2004  Elsevier Science B.V.
File 144:Pascal 1973-2004/Oct W2
         (c) 2004 INIST/CNRS
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         (c) format only 2004 The Dialog Corp.
File 162:Global Health 1983-2004/Sep
         (c) 2004 CAB International
File 266:FEDRIP 2004/Jul
         Comp & dist by NTIS, Intl Copyright All Rights Res
File 467:ExtraMED(tm) 2000/Dec
         (c) 2001 Informania Ltd.
```

18/5/3 **(Item 3 from file: 5)**
DIALOG(R)File 5:Biosis Previews(R)

0010737483 BIOSIS NO.: 199799371543
Radiation-induced differentiation of human skin fibroblasts: Relationship with cell survival and collagen production
AUTHOR: Lara P C; Russell N S (Reprint); Smolders I J H; Bartelink H; Begg A C; Coco-Martin J M
AUTHOR ADDRESS: Dep. Radiotherapy, Netherlands Cancer Inst., Plesmanlaan 121, 1066 CX Amsterdam, Netherlands**Netherlands
JOURNAL: International Journal of Radiation Biology 70 (6): p683-692 1996 1996
ISSN: 0955-3002
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The aim of this study was to determine whether significant inter-individual differences exist between skin fibroblast strains obtained from radiotherapy patients in both radiation-induced differentiation and collagen production in vitro, for use as potential parameters for a predictive assay for fibrosis following radiotherapy in patients. Morphological cell differentiation was determined 7 days after irradiation in seven early-passage primary human fibroblast cell strains and correlated with cell survival. Collagen production was measured in two cell strains by **flow** cytometry and incorporation of 3H-proline. There was a wide variation in the extent of radiation-induced differentiation for the seven cell strains, each showing a dose-related increase. The correlation between induced differentiation and cell survival was poor (r = 0.64) but statistically significant (p lt 0.01). Collagen synthesis increased 7 days after irradiation for one cell strain (HF-48), as measured by incorporation of 3H-proline, but not in radiation sensitive AT-1 cells. The **collagen I** content of the two cell strains was assessed by **flow** cytometry but no significant differences were observed between the strains tested or with increasing dose. In conclusion, marked variations in radiation-induced fibroblast differentiation were observed between patients, this being an important criterion for a predictive assay.

DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Cell Biology; Development; Integumentary System--Chemical Coordination and Homeostasis; Radiation Biology
BIOSYSTEMATIC NAMES: Hominidae--Primates, Mammalia, Vertebrata, Chordata, Animalia
ORGANISMS: human (Hominidae)
COMMON TAXONOMIC TERMS: Animals; Chordates; Humans; Mammals; Primates; Vertebrates
MISCELLANEOUS TERMS: AT-1 CELL LINE; CELL SURVIVAL; COLLAGEN PRODUCTION ; HF-48 CELL LINE; HUMAN SKIN FIBROBLAST CELLS; INTEGUMENTARY SYSTEM; PREDICTIVE ASSAY DEVELOPMENT; RADIATION BIOLOGY; RADIATION SENSITIVE; RADIATION-INDUCED DIFFERENTIATION; RADIOTHERAPY; RADIOTHERAPY PATIENT; SKIN FIBROBLASTS; THERAPEUTIC METHOD
CONCEPT CODES:
02508 Cytology - Human
06502 Radiation biology - General
10060 Biochemistry studies - General
18501 Integumentary system - General and methods
25502 Development and Embryology - General and descriptive
BIOSYSTEMATIC CODES:
86215 Hominidae

01431177 ORDER NO: AADAA-I9527963

CELL-MATRIX INTERACTIONS OF MICROVESSEL ENDOTHELIAL CELLS IN RESPONSE TO BASIC FIBROBLAST GROWTH FACTOR

Author: HOYING, JAMES B.
Degree: PH.D.
Year: 1994
Corporate Source/Institution: THE UNIVERSITY OF ARIZONA (0009)
Director: STUART K. WILLIAMS
Source: VOLUME 56/05-B OF DISSERTATION ABSTRACTS INTERNATIONAL.
PAGE 2419. 233 PAGES
Descriptors: BIOLOGY, CELL; BIOLOGY, PHYSIOLOGY
Descriptor Codes: 0379; 0433

Vertebrate tissues consist of parenchyma and vascular elements all of which are necessary for the specific form and function of these tissues. In a unique process termed angiogenesis, vessels invade forming tissues to provide for proper tissue **perfusion** . Much is known about the molecular and cellular elements of angiogenesis, however, it is not clear how these elements are coordinated to produce specific microvascular beds. In an effort to answer this question, the effects of basic **fibroblast growth** factor (bFGF) on human microvessel endothelial cell (HMVEC) interactions with **collagen I** were examined. HMVEC migration on **collagen I** was chosen as the model angiogenic response. Utilizing two distinct migration assays, bFGF either induced migration or had no effect. Examination of HMVEC adhesion with two separate assays revealed that HMVEC adhesion to **collagen I** was altered by bFGF treatment and depended on the density of HMVEC at the time of treatment. Adhesion of HMVEC with or without bFGF treatment was mediated entirely by β1 integrins as demonstrated with a blocking antibody studies. Experiments were performed to determine the mechanism by which bFGF can alter HMVEC adhesion and focused on low density HMVEC. The reduction in adhesion of low density HMVEC following bFGF treatment correlated with no change in β1 integrin surface expression, delayed cell spreading, altered organization of β1 integrin into substrate contacts, and serine/threonine phosphorylation of the β1 subunit. To evaluate the coordinated effects of bFGF on angiogenesis, an in vitro model simulating a microvascular environment was developed utilizing isolated microvessel fragments from rat adipose tissue cultured in three dimensional **collagen I** gels. The addition of crude basic **fibroblast growth** factor to the **cultures** resulted in the **growth** of significantly longer microvessels and the expression of an endothelial cell protein, von Willebrand factor. Based on this work, it is apparent that cellular responses to physiological signals during angiogenesis are multifactorial and are sensitive to many coincidental environmental factors such as cell density. The influence of these environmental factors is such as to substantially alter the effects of a signalling factor acting alone.

18/5/9 (Item 2 from file: 73)
DIALOG(R)File 73:EMBASE

10766261 EMBASE No: 2000246591

Characteristics of human chondrocytes, osteoblasts and fibroblasts seeded onto a type I/III collagen sponge under different culture conditions. A light, scanning and transmission electron microscopy study

Fuss M.; Ehlers E.-M.; Russlies M.; Rohwedel J.; Behrens P.
E.-M. Ehlers, Institut fur Anatomie, Medizinischen Universitat zu Lubeck, Ratzeburger Allee 160, D-23538 Lubeck Germany

Annals of Anatomy (ANN. ANAT.) (Germany) 2000, 182/4 (303-310)
CODEN: ANANE ISSN: 0940-9602
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 25

Hyaline cartilage has only a limited capacity of regeneration, thus, lesions of articular cartilage can lead to early osteoarthrosis. Current concepts in conservative orthopedic therapy do not always lead to satisfying results. As one new attempt to facilitate cartilage repair, autologous transplantation of articular chondrocytes is investigated in different assays. This study was designed to create a resistible and stable cell-matrix-biocomposite with viable and biosynthetically active human chondrocytes, osteoblasts or fibroblasts. This biocomposite might serve as an implant to treat deep osteochondral defects in the knee. We collected cartilage, spongiosa and skin probes from healthy patients undergoing hip-surgery and enzymatically liberated the chondrocytes, seeded them into culture flasks and cultured them until confluent. The spongiosa and the skin samples were also placed in culture flasks and cells cultured until confluent. After 4-6 weeks, cells were trypsinized and grown on a type I/III collagen matrix (Chondrogide(TM), Geistlich Biomaterials, Wolhusen, Switzerland) for 7 days in standard Petri dishes and in a special **perfusion** chamber culture system. As controls, cells were seeded onto plastic surfaces. Then scaffolds were fixed and embedded for light microscopy and electron microscopy by routine methods. Light microscopically, chondrocytes grown on the surface of the scaffold form clusters or a dense layer of sometimes rather fibroblast-like and sometimes roundish, chondrocyte-like cells. Only a few cells grow deeper into the matrix. In transmission electron microscopy, the cells have a rather chondrocyte-like morphology which emphasizes the matrix-induced redifferentiation after dedifferentiation of chondrocytes in monolayer-culture in culture flasks. Chondrocytes on plastic surfaces have a spinocellular aspect with little signs of differentiation. Grown on Chondrogide(TM), cells are more roundish and adhere firmly to the collagen fibrils of the scaffold. Osteoblasts grown on the collagen scaffold and examined by light microscopy form a thin cell-layer on the surface of the matrix with a reticular layer of dendritic cells underneath this sheet. Transmisson electron micrographs show spinocellular and flat cells on the collagen fibrils. Scanning electron micrographs show large dendritic osteoblasts on plastic and a confluent layer of flattened, dendritic cells on the collagen scaffold. Fibroblasts form a thick multi-layer of typical spinocellular cells on the **collagen** matrix. **Fibroblasts grown** on plastic surfaces and examined by scanning electron microscopy also show a dense layer of fibroblast-like cells. For all three different types of cells no morphological differences could be seen when comparing cultivation in the **perfusion** culture system to cultivation in standard Petri dishes, although mechanical stress is believed to induce differentiation of chondrocytes. Especially the observed partially differentiated chondrocyte-matrix biocomposite might serve as an implant to treat deep cartilage defects, whereas osteoblasts and fibroblasts seem to be less suited.

DEVICE BRAND NAME/MANUFACTURER NAME: Chondrogide/geistlich biomaterials/ Switzerland
DEVICE MANUFACTURER NAMES: geistlich biomaterials/Switzerland
DRUG DESCRIPTORS:

* **collagen type** 1; * **collagen type** 3
collagen fibril
MEDICAL DESCRIPTORS:
*cartilage cell; *cell culture; *fibroblast culture; *osteoblast
biocompatibility; cell structure; microscopy; scanning electron microscopy;
sponge; transmission electron microscopy; human; human cell; article
SECTION HEADINGS:
  001 Anatomy, Anthropology, Embryology and Histology
  027 Biophysics, Bioengineering and Medical Instrumentation
  033 Orthopedic Surgery

18/5/14 (Item 1 from file: 144)
DIALOG(R)File 144:Pascal

12800719 PASCAL No.: 97-0013593
Tissue - engineered **heart valves : Autologous valve leaflet replacement study in a lamb model**
Cardiovascular surgery 1995
SHINOKA T; MA P X; SHUM-TIM D; BREUER C K; CUSICK R A; ZUND G; LANGER R; VACANTI J P; MAYER J E JR
WEISEL Richard D, ed
Department of Cardiovascular Surgery, Children's Hospital, Boston, Mass, United States; Department of Surgery, Children's Hospital, Boston, Mass, United States; Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, United States
American Heart Association. Council on Cardio-Thoracic and Vascular Surgery, United States.
Council on Cardio-Thoracic and Vascular Surgery. American Heart Association. Scientific Sessions, 68 (Anaheim, California USA) 1995-11-13
Journal: Circulation : (New York), 1996, 94 (9 SUP) 164-168
ISSN: 0009-7322 CODEN: CIRCAZ Availability: INIST-5907; 354000066840970300
No. of Refs.: 13 ref.
Document Type: P (Serial); C (Conference Proceedings) ; A (Analytic)
Country of Publication: United States
Language: English
Background We have previously reported the successful creation of **tissue - engineered** valve leaflets and the implantation of these autologous tissue leaflets in the pulmonary valve position. This study was designed to trace cultured cells that were **seeded** onto a biodegradable polymer with the use of a 1,1'-dioctadecyl-3,3,3',3'-tetramethylindo-carbocyanine perchlorate (Di-I) cell-labeling method. We also examined the time-related biochemical, biomechanical, and histological characteristics and evolution of these tissue constructs. Methods and Results Mixed cell populations of endothelial cells and fibroblasts were isolated from explanted ovine arteries. Endothelial cells were selectively labeled with an acetylated low-density lipoprotein marker and separated from fibroblasts with the use of a fluorescence-activated cell sorter. A synthetic biodegradable **scaffold** consisting of polyglycolic acid fibers was **seeded** first with fibroblasts, then coated with endothelial cells. Using these methods, we implanted autologous cell/polymer constructs in six animals. In two additional control animals, a leaflet of polymer was implanted without prior cell **seeding** . In each animal, cardiopulmonary bypass was used to completely resect the right posterior leaflet of the pulmonary valve and replace it with an engineered valve leaflet with (n=6) or without (n=2) prior cultured cell **seeding** . The animals were killed either after 6 hours or after 1, 6, 7, 9, or 11 weeks, and the implanted valve leaflets were examined histologically, biochemically, and biomechanically. 4-Hydroxyproline assays were performed to determine **collagen** content. Leaflet strength was evaluated in vitro with a mechanical tester. Factor VIII and elastin stains were done to verify histologically that endothelial cells and elastin, respectively, were present. Animals receiving leaflets made from polymers without cell **seeding** were killed and examined in a similar fashion after 8 weeks. In the control animals, the **acellular** polymer leaflets were completely degraded, with no residual leaflet tissue at 8 weeks. The **tissue - engineered** valve leaflet persisted in each animal in the experimental group. 4-Hydroxyproline analysis of the constructs showed a progressive increase in **collagen** content. Immunohistochemical staining demonstrated elastin fibers in the **matrix** and factor VIII on the surface of the leaflet. The cell-labeling experiments demonstrated that the cells on the leaflets had persisted from the in vitro **seeding** of the leaflets. Conclusions In the **tissue - engineered** heart valve leaflet, transplanted autologous cells generated a proper **matrix** on the polymer **scaffold** in a physiological environment at a period of 8 weeks after implantation.

English Descriptors: Pulmonary valve; **Seeding** ; Endothelial cell;

Fibroblast ; Cell **culture** ; Surgery; Replacement; Immunohistochemistry; Follow up study; Treatment; Sheep; Animal model

Broad Descriptors: Artiodactyla; Ungulata; Mammalia; Vertebrata; Pathology; Artiodactyla; Ungulata; Mammalia; Vertebrata; Anatomopathologie; Artiodactyla; Ungulata; Mammalia; Vertebrata; Anatomia patologica

French Descriptors: Valvule pulmonaire; Ensemencement; Cellule **endotheliale** ; **Fibroblaste** ; **Culture cellulaire** ; Chirurgie; Remplacement; Immunohistochimie; Etude longitudinale; Traitement; Mouton; Modele animal

Classification Codes: 002B25E

```
Set     Items   Description
S1      47621   MYOFIBROBLAST? OR FIBROBLAST? OR ENDOTHELIAL?
S2       1304   ACELLULAR? OR DECELLULAR? OR DE()CELLULAR?
S3     582868   MATRIX? OR LEAFLET()INTERSTITIAL()TISSUE? OR SEEDING OR FR-
             AME? OR SCAFFOLD? OR MESH? OR GRAFT? OR ALLOGRAFT? OR SEEDED -
             OR SEEDS OR SEED
S4      39840   COLLAGEN?
S5       2196   TISSUE()ENGINEER? OR BIOPROSTHET?
S6    1210489   CULTUR? OR GROW? OR PRODUC? OR CLONE? OR REPLICAT? OR CLON-
             ING OR REPRODUC?
S7     688456   PULSAT? OR FLOW? OR PERFUS?
S8     285325   CARDIAC? OR VALV? OR MITRAL? OR PULMONAR? OR TRICUSPID? OR
             HEART? OR CARDIOLOG?
S9       2468   S1(10N)S6(10N)S4
S10      1225   S1(2N)S6(5N)S4
S11        27   S9(S)S3(S)S2
S12        25   S9(10N)S7
S13        10   S10(10N)(S2 OR S3)(10N)(S8 OR S5)
S14        14   (S11 OR S12 OR S13) AND IC=A61F?
S15        14   IDPAT (sorted in duplicate/non-duplicate order)
S16       759   S1(2N)S6(3N)S4
S17      3480   COLLAGEN(N)TYPE? OR COLLAGEN()(ONE OR 1)
S18        53   S16(S)S17
S19        31   S16(5N)S17
S20         5   S19 AND IC=A61F?
S21        16   S20 OR S15
S22         0   S19(5N)S7
S23         3   S19(S)S7
S24        19   S23 OR S21
S25        19   IDPAT (sorted in duplicate/non-duplicate order)
S26        19   IDPAT (primary/non-duplicate records only)
File 348:EUROPEAN PATENTS 1978-2004/Oct W01
         (c) 2004 European Patent Office
File 349:PCT FULLTEXT 1979-2002/UB=20041014,UT=20041007
         (c) 2004 WIPO/Univentio
```

26/3,K/1 **(Item 1 from file: 348)**
DIALOG(R)File 348:EUROPEAN PATENTS

01781295
Treated tissue for implantation and preparation methods
Vorbehandeltes Gewebe fur die Implantation und ein Verfahren
Tissu traite pour l'implantation et une methode de preparation
PATENT ASSIGNEE:
CRYOLIFE, INC, (1003661), 2211 New Market Parkway, Suite 142, Marietta, GA 30067, (US), (Applicant designated States: all)
INVENTOR:
Goldstein, Steven, 2939 Greenbrook Way, Atlanta GA 30345, (US)
LEGAL REPRESENTATIVE:
Baldock, Sharon Claire et al (73341), BOULT WADE TENNANT, Verulam Gardens 70 Gray's Inn Road, London WC1X 8BT, (GB)
PATENT (CC, No, Kind, Date): EP 1452153 A1 040901 (Basic)
APPLICATION (CC, No, Date): EP 2004009948 950227;
PRIORITY (CC, No, Date): US 213754-940314
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE
RELATED PARENT NUMBER(S) - PN (AN):
EP 871414 (EP 95911929)
INTERNATIONAL PATENT CLASS: **A61F-002/24** ; A61L-027/54; A61L-027/38
ABSTRACT WORD COUNT: 123
NOTE:
Figure number on first page: NONE

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	200436	2497
SPEC A	(English)	200436	7911
Total word count - document A			10408
Total word count - document B			0
Total word count - documents A + B			10408

INTERNATIONAL PATENT CLASS: **A61F-002/24** ...

...SPECIFICATION collagen types, and the phenotype appears to be imposed by specific tissue environments; i.e., **cultured fibroblasts** synthesize **collagen types** according to their site of origin. Fibroblasts also produce various glycosaminoglycans and fibronectin and growth...

...CLAIMS heart valve tissue, and wherein the cellular repopulation step is conducted by incubating the tissue **matrix** in a nutrient environment and in the presence of **fibroblast** cells and an effective amount of **fibroblast growth** factor.

31. A process for generating xenogeneic implant or **grafts** from non-human **collagenous** , connective, or vascular tissue wherein the natural tissue is **decellularized** and washed to remove cellular and/or extracellular antigens, followed by treatment of the tissue **matrix** with adhesion factors comprised of fibronectin and heparin effective to promote attachment thereto of fibroblast cells immunologically acceptable to the implant or **graft** recipient, wherein the tissue **matrix** treated with adhesion factor is repopulated by incubating the **matrix** in the presence of the fibroblast cells and fibroblast growth factor until such cellular repopulation...

26/3,K/4 (Item 4 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

00976063
PREPARATION OF COLLAGEN
HERSTELLUNG VON KOLLAGEN
PREPARATION DE COLLAGENE
PATENT ASSIGNEE:
Nova Gen, Inc., (2590950), 5686 Geranium Court, Newark, CA 94560, (US),
(Proprietor designated states: all)
INVENTOR:
GUNASEKARAN, Subramanian, 5686 Geranium Court, Newark, CA 94560, (US)
LEGAL REPRESENTATIVE:
Glawe, Delfs, Moll & Partner (100691), Patentanwalte Rothenbaumchaussee 58, 20148 Hamburg, (DE)
PATENT (CC, No, Kind, Date): EP 1007065 A1 000614 (Basic)
EP 1007065 B1 040421
WO 1998030225 980716
APPLICATION (CC, No, Date): EP 98902442 980112; WO 98US371 980112
PRIORITY (CC, No, Date): US 782138 970113
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE
INTERNATIONAL PATENT CLASS: A61K-038/17; **A61F-002/00** ; C12P-021/06; C07K-014/78; A61L-027/00; A61L-015/40; A61P-007/04
NOTE:
No A-document published by EPO
LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS B	(English)	200417	657
CLAIMS B	(German)	200417	645
CLAIMS B	(French)	200417	785
SPEC B	(English)	200417	14562
Total word count - document A			0
Total word count - document B			16649
Total word count - documents A + B			16649

...INTERNATIONAL PATENT CLASS: **A61F-002/00**

...SPECIFICATION is characterized as being densely packed, thick fibrils with marked variation in diameter. It is **produced** by **fibroblasts** , osteoblasts, odontoblasts, and chondroblasts.
Collagen type II is primarily found in cartilage (e.g., hyaline and elastic cartilages). The primary function...

26/3,K/15 **(Item 15 from file: 349)**
DIALOG(R)File 349:PCT FULLTEXT

00357661
STROMAL CELL-BASED THREE-DIMENSIONAL CULTURE SYSTEM FOR FORMING TUBES, TENDONS, LIGAMENTS AND CORRECTIVE STRUCTURES
SYSTEME DE CULTURE TRIDIMENSIONNEL BASE SUR LES CELLULES DU STROMA ET DESTINE A LA FORMATION DE TUBES, DE TENDONS, DE LIGAMENTS ET DE STRUCTURES CORRECTIVES
Patent Applicant/Assignee:
ADVANCED TISSUE SCIENCES INC,
Inventor(s):
NAUGHTON Gail K,
NAUGHTON Brian A,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9640175 A1 19961219
Application: WO 96US8425 19960603 (PCT/WO US9608425)
Priority Application: US 95749 19950607
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AU CA JP KR NZ AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE
Publication Language: English
Fulltext Word Count: 15707
...International Patent Class: **A61F-02:02** ...

... **A61F-02:04**
Fulltext Availability:
Detailed Description

Detailed Description
... The embodiment of the invention provides for the placement of stromal tissue under mechanical or **pulsatile** forces to alter the formation and alignment of **collagen** fibers into bundles more dense and parallel than those routinely seen in dermis. By placing dermal **fibroblasts** on polymers and **growing** the tissues under increasing pulsing mechanical force, the final structure will have the tensile strength...

26/3,K/16 **(Item 16 from file: 349)**
DIALOG(R)File 349:PCT FULLTEXT

00325705 **Image available**
THREE-DIMENSIONAL HUMAN CELL CULTURES ON CARDIAC VALVE FRAMEWORKS AND THEIR USES
CULTURES TRIDIMENSIONNELLES DE CELLULES HUMAINES SUR DES STRUCTURES DE VALVULES CARDIAQUES, ET LEURS UTILISATIONS
Patent Applicant/Assignee:
ADVANCED TISSUE SCIENCES INC,
Inventor(s):
NAUGHTON Gail K,
NAUGHTON Brian A,
PURCHIO Anthony F,
LANDEEN Lee K,
ZELTINGER Joan,
CAMPBELL Todd D,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9608213 A1 19960321
Application: WO 95US11395 19950908 (PCT/WO US9511395)
Priority Application: US 9462 19940912; US 95165 19950607
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AM AU BB BG BR BY CA CN CZ EE FI GE HU IS JP KG KP KR KZ LK LR LT LV MD MG MK MN MX NO NZ PL RO RU SG SI SK TJ TM TT UA UZ VN KE MW SD SZ UG AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
Publication Language: English
Fulltext Word Count: 13037

Main International Patent Class: **A61F-002/24**
Fulltext Availability:
Detailed Description

Detailed Description
... and steam
sterilization including, but not limited to autoclaving.

No viable cells remain in the **decellularized** /sterilized tissue samples which are used as a **scaffold** or **framework** for **culturing** the stromal cells.

The stromal cells which are inoculated onto the **scaffold** , may include dermal or **cardiac** **fibroblasts** , and/or cells capable of **producing** **collagen** types I and III, and in some instances, elastin, which are typically **produced** in **heart** **valves** , (See Table I), The stromal cells and connective tissue proteins naturally secreted by the stromal cells attach to and substantially envelope the three-dimensional **framework** or construct, having interstitial spaces bridged by the stromal cells, The living stromal tissue so...inflammatory reactions in vivo, and 2) escape immunological surveillance and rejection.

Stromal tissue comprising dermal **fibroblasts** , **cardiac** **fibroblasts** and cells capable of **producing** **collagen** **type** I and III, elastin and other **heart** **valve** **matrix** proteins, for example, but not limited to fibronectin and glycosaminoglycans, are used to grow in...

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S1      12910   MYOFIBROBLAST? OR FIBROBLAST? OR ENDOTHELIAL?
S2        261   ACELLULAR? OR DECELLULAR? OR DE()CELLULAR?
S3    1438458   MATRIX? OR LEAFLET()INTERSTITIAL()TISSUE? OR SEEDING OR FR-
            AME? OR SCAFFOLD? OR MESH? OR GRAFT? OR ALLOGRAFT? OR SEEDED -
            OR SEEDS OR SEED
S4      16060   COLLAGEN?
S5        775   TISSUE()ENGINEER? OR BIOPROSTHET?
S6    3793903   CULTUR? OR GROW? OR PRODUC? OR CLONE? OR REPLICAT? OR CLON-
            ING OR REPRODUC?
S7    1444347   PULSAT? OR FLOW? OR PERFUS?
S8     868971   CARDIAC? OR VALV? OR MITRAL? OR PULMONAR? OR TRICUSPID? OR
            HEART? OR CARDIOLOG?
S9       1513   S1 AND S6 AND S4
S10       761   S1(2N)S6 AND S4
S11        28   S9 AND S3 AND S2
S12       149   S9 AND S7
S13       127   S10 AND (S2 OR S3) AND (S8 OR S5)
S14        32   (S11 OR S13) AND IC=A61F?
S15     15545   MC=(B04-C02E2? OR B04-F0200E? OR B04-H06G? OR B04-H20A? OR
            B04-N0200E? OR B11-C04A? OR D09-C01C?)
S16        59   (S11 OR S13) AND S15
S17        48   S8 AND S16
S18        64   S17 OR S14
S19        17   S18 NOT AD=20000704:20020704
S20        10   S19 NOT AD=20020704:20041020
S21        36   S14 OR S20
S22        36   IDPAT (sorted in duplicate/non-duplicate order)
S23        36   IDPAT (primary/non-duplicate records only)
File 347:JAPIO Nov 1976-2004/Jun(Updated 041004)
         (c) 2004 JPO & JAPIO
File 350:Derwent WPIX 1963-2004/UD,UM &UP=200466
         (c) 2004  Thomson Derwent
```

23/5/3 **(Item 3 from file: 350)**
DIALOG(R)File 350:Derwent WPIX

016261622
WPI Acc No: 2004-419516/200439
Related WPI Acc No: 2002-238812
XRAM Acc No: C04-157463
XRPX Acc No: N04-333004

Isolated myofibroblast **for use in** bioprosthetic heart valve **is genetically altered to increase type I** collagen production **relative to type III** collagen production

Patent Assignee: HOFFMAN-KIM D (HOFF-I); HOPKINS R A (HOPK-I)
Inventor: HOFFMAN-KIM D; HOPKINS R A
Number of Countries: 001 Number of Patents: 001
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week	
US 20040106991	A1	20040603	US 2000195673	P	20000407	200439	B
			US 2001828768	A	20010409		
			US 2003722279	A	20031124		

Priority Applications (No Type Date): US 2000195673 P 20000407; US 2001828768 A 20010409; US 2003722279 A 20031124
Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
US 20040106991	A1		17	A61F-002/24	Provisional application US 2000195673
					Div ex application US 2001828768
					Div ex patent US 6652583

Abstract (Basic): US 20040106991 A1

NOVELTY - Isolated **myofibroblast** is genetically altered to increase type I **collagen production** relative to type III **collagen production** .

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:

(1) a **bioprosthetic heart valve** comprising an **acellular matrix** and isolated **myofibroblasts** in which at least 60 % of the total **collagen produced** by the **myofibroblasts** is type I **collagen** ;

(2) enhancing viability and contractile activity of **myofibroblasts** in vitro, which comprises **culturing** the **myofibroblast** under pulsatile flow conditions;

(3) enhancing **production** of type I **collagen** by an isolated **myofibroblast** , which comprises **culturing** the **myofibroblast** under pulsatile flow conditions; and

(4) manufacturing an artificial **heart valve** , which comprises providing an **acellular matrix** , **seeding** the **matrix** with isolated **myofibroblasts** , and **culturing** the **myofibroblasts** under pulsatile flow conditions.

USE - The isolated **myofibroblast** is used in a **bioprosthetic heart valve** (claimed).

ADVANTAGE - The use of the inventive isolated **myofibroblast** provides a **bioprosthetic heart valve** eliminates the need for immune suppression when transplanting cells from a donor and eliminates biocompatibility concerns which accompany the use of biomaterials in **tissue engineered valves** .

pp; 17 DwgNo 0/6

Title Terms: ISOLATE; **MYOFIBROBLAST** ; **HEART** ; **VALVE** ; GENETIC; ALTER; INCREASE; TYPE; **COLLAGEN** ; **PRODUCE** ; RELATIVE; TYPE; **COLLAGEN** ; **PRODUCE**
Derwent Class: B04; D22; P32
International Patent Class (Main): **A61F-002/24**
File Segment: CPI; EngPI

23/5/4 (Item 4 from file: 350)
DIALOG(R)File 350:Derwent WPIX

016070585
WPI Acc No: 2004-228446/200422
XRAM Acc No: C04-089878
XRPX Acc No: N04-180582

In vitro preparation of homologous heart valves, useful for replacement of diseased valves, by inoculating biodegradable carrier with fibroblasts and attachment to a non-degradable stent

Patent Assignee: SYMETIS AG (SYME-N)
Inventor: GREGOR Z; PHILIPP H S; HOERSTRUP S P; ZUEND G
Number of Countries: 101 Number of Patents: 003
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week	
DE 10235237	A1	20040212	DE 10235237	A	20020801	200422	B
WO 200418008	A1	20040304	WO 2002EP9906	A	20020904	200422	
AU 2002347023	A1	20040311	AU 2002347023	A	20020904	200457	

Priority Applications (No Type Date): DE 10235237 A 20020801
Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
DE 10235237	A1		12	C12N-005/08	
WO 200418008	A1 G			A61L-027/38	

Designated States (National): AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW
Designated States (Regional): AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW

AU 2002347023	A1		A61L-027/38	Based on patent WO 200418008

Abstract (Basic): DE 10235237 A1

NOVELTY - An in vitro method for preparing homologous heart valves (A), is new.

DETAILED DESCRIPTION - An in vitro method for preparing homologous heart valves (A) comprises:

(a) preparation of a biodegradable carrier (**scaffold**);

(b) **seeding** the carrier with homologous **fibroblasts** and/or **myofibroblasts** to form a connective tissue **matrix** ;

(c) optionally **seeding** the **matrix** with **endothelial** cells and

(d) fixing the **matrix** to a **framework** (stent) that is not (easily) degraded.

The **matrix** , before and/or after fixing, is introduced into a pulsatile flow chamber and the flow rate in the chamber increased (dis)continuously.

INDEPENDENT CLAIMS are also included for:

(1) similar method in which the carrier and stent are fixed together before **seeding** with cells;

(2) autologous heart valves prepared by the method; and

(3) autologous heart valves having a connective tissue inner structure, surrounded by a layer of **endothelial** cells and fixed to a stent.

USE - The method is used to make heart valves for replacement of diseased valves.

ADVANTAGE - (A) Can withstand the flow conditions in the human heart and can be implanted simply, specifically without requiring suturing through the connective tissue core of the valve.

pp; 12 DwgNo 0/3

Title Terms: VITRO; PREPARATION; HOMOLOGUE; HEART; VALVE; USEFUL; REPLACE; DISEASE; VALVE; INOCULATE; BIODEGRADABLE; CARRY; **FIBROBLAST** ; ATTACH; NON; DEGRADE; STENT
Derwent Class: A96; B04; D16; D22; P32; P34
International Patent Class (Main): A61L-027/38; C12N-005/08
International Patent Class (Additional): **A61F-002/24** ; A61L-027/18; A61L-027/50; A61L-031/00

File Segment: CPI; EngPI

23/5/9 (Item 9 from file: 350)
DIALOG(R)File 350:Derwent WPIX

015737570
WPI Acc No: 2003-799771/200375
Related WPI Acc No: 2002-680810; 2004-154432
XRAM Acc No: C03-220827

Generation of transplantable recellularized and reendothelialized vascular tissue graft **by recellularizing tissue** matrix **with (myo)** fibroblastic **cell population and reendothelializing with** endothelial **cell population**

Patent Assignee: BIOSCIENCE CONSULTANTS LLC (BIOS-N)
Inventor: WOLFINBARGER L
Number of Countries: 001 Number of Patents: 001
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week	
US 6432712	B1	20020813	US 99166884	P	19991122	200375	B
			US 2000595717	A	20000616		

Already applied

Priority Applications (No Type Date): US 99166884 P 19991122; US 2000595717 A 20000616
Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
US 6432712	B1		19	C12N-005/00	Provisional application US 99166884

Abstract (Basic): US 6432712 B1

NOVELTY - Generating a transplantable recellularized and reendothelialized tissue **graft** comprises:

(i) recellularizing treated tissue **matrix** with **fibroblastic** or **myofibroblastic** cell population;

(ii) reendothelializing a luminal surface of the recellularized **matrix** with an **endothelial** cell population; and

(iii) preconditioning the repopulated tissue **matrix** .

DETAILED DESCRIPTION - Generating a transplantable recellularized and reendothelialized tissue **graft** comprises:

(i) harvesting tissue from a donor or **producing** tissue by **tissue engineering** methods;

(ii) eliminating native cells to provide a tissue **matrix** ;

(iii) removing basement membrane from a luminal surface of the tissue **matrix** ;

(iv) treating the tissue **matrix** with factors;

(v) recellularizing the treated tissue **matrix** with a **fibroblastic** or **myofibroblastic** cell population;

(vi) reendothelializing a luminal surface of the recellularized tissue **matrix** with an **endothelial** cell population; and

(vii) preconditioning the repopulated tissue **matrix** to promote cell to cell communication, retard apoptotic events, and orient molecular structure of the repopulated tissue **matrix** to **produce** the transplantable recellularized and reendothelialized tissue **graft** .

USE - **Production** of a repopulated, reendothelialized vascular tissue **graft** . The tissue may be an artery or vein, a small intestine, a urethra or an aortic or **pulmonary heart valve** which is cellular or **acellular** at the time of processing. The vascular tissue is derived from human or non-human donors (all claimed). The tissue **graft** is used in replacement of defective tissues e.g. defective **heart valves** and vascular conduits.

ADVANTAGE - The process is carried out in a closed system that restricts contamination by microbiological and chemical/biological elements.

pp; 19 DwgNo 0/13

Title Terms: GENERATE; VASCULAR; TISSUE; **GRAFT** ; TISSUE; **MATRIX** ; MYO; CELL; POPULATION; ENDOTHELIUM; CELL; POPULATION
Derwent Class: A96; B04; D16; D22
International Patent Class (Main): C12N-005/00
File Segment: CPI

23/5/14 (Item 14 from file: 350)
DIALOG(R)File 350:Derwent WPIX

015178724 **Image available**
WPI Acc No: 2003-239254/200323
XRAM Acc No: C03-061353
XRPX Acc No: N03-190627
Human engineered tissue-type heart valve comprising several assembled leaflets each of which comprises at least five layers of a living tissue sheet fused together to form a self-supporting human engineered tissue
Patent Assignee: ALTERTEK BIO INC (ALTE-N); EDWARDS LIFESCIENCES CORP (EDWA-N)
Inventor: AUGER F; BERGERON F; GERMAIN L; LAFRANCE H; ROBERGE C
Number of Countries: 101 Number of Patents: 004
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week
WO 200307795	A2	20030130	WO 2002US22836	A	20020716	200323 B
US 20030027332	A1	20030206	US 2001306058	P	20010716	200325
			US 2002198628	A	20020716	
EP 1406561	A2	20040414	EP 2002750139	A	20020716	200426
			WO 2002US22836	A	20020716	
AU 2002320610	A1	20030303	AU 2002320610	A	20020716	200452

Priority Applications (No Type Date): US 2001306058 P 20010716; US 2002198628 A 20020716
Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
WO 200307795	A2	E	74	A61B-000/00	

Designated States (National): AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG UZ VN YU ZA ZM ZW
Designated States (Regional): AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW

US 20030027332	A1		A61K-048/00	Provisional application US 2001306058
EP 1406561	A2	E	A61F-002/24	Based on patent WO 200307795

Designated States (Regional): AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI SK TR

AU 2002320610	A1		A61B-000/00	Based on patent WO 200307795

Abstract (Basic): WO 200307795 A2

NOVELTY - A human engineered tissue (HT)-type **heart valve** (50) comprising several leaflets assembled to form a **heart valve**, where each leaflet comprises at least five layers of at least one living tissue sheet fused together to form a self-supporting HT, is new. Each leaflet is comprised of layers of at least one living tissue sheet fused together to form a self-supporting HT having a thickness of at least 0.16 mm.

DETAILED DESCRIPTION - A human engineered tissue (HT)-type **heart valve** comprising several leaflets assembled to form a **heart valve**, where each leaflet comprises at least five layers of at least one living tissue sheet fused together to form a self-supporting HT. Each leaflet is comprised of layers of at least one living tissue sheet fused together to form a self-supporting HT having a thickness of at least 0.16 mm. Each leaflet is comprised of layers of at least allogeneic living tissue sheet fused together to form a self supporting HT which undergoes living cell replacement upon implantation in a patient so that at least some of the allogeneic cells are replaced with the patient's living cells. The HT-type **heart valve** comprises a tissue leaflet subassembly mated with wireform to form a **heart valve**, where each leaflet is comprised of at least five layers of at least one living tissue sheet fused together to form self-supporting HT and where at least a portion of the wireform is covered with the tissue.

INDEPENDENT CLAIMS are also included for:

(1) making (M1) a human engineered **heart valve**, comprising:

(a) generating at least one living tissue sheet by secreting an extracellular **matrix** from cells;

(b) layering at least one living tissue sheet to form a layered construct having at least seven layers; and

(c) culturing the layered construct to the fuse the layers to form a HT; and

(2) preparing (M2) HT for use in making a **heart valve** , comprising:

(a) generating at least one living tissue sheet by secreting an extracellular **matrix** from cells;

(b) layering at least one living tissue sheet to form a layered construct;

(c) culturing the layered construct to fuse the layers to form the HT; and

(d) regulating shrinkage of the HT, the method optionally involves generating at least one living tissue sheet by secreting an extracellular **matrix** from cells, layering at least one living tissue sheet to form a layered construct, culturing the layered construct to fuse the layers to form HT, and cutting a leaflet shape out of the HT which is dimensionally larger than a desired leaflet shape to account for shrinkage.

USE - (I) is useful for **heart valve** replacement procedures.

ADVANTAGE - The leaflets of the **heart valve** are formed from self-supporting HT. The self-supporting tissue is comprised of living biological cells and extracellular **matrix** without the presence of nonviable exogenous **scaffolding** structures. The living **valve** implies responsive and self-renewing tissue and inherent healing potential, its biological **matrix** can be remodeled by the body according to the needs of the environment, and the absence of the synthetic **scaffolding** tissue preclude foreign body reaction, allowing complete **valve** integration and limit **valve** infection. The **tissue engineered heart valve** consists of totally living human tissue theoretically function like a native biological structure with the potential to grow, to repair and to remodel. The **valve** would remodel into a human living **valve** and adapt to its new environment, such as supporting human body's growth from infant to adult.

DESCRIPTION OF DRAWING(S) - The figure shows the top-view of a living **tissue - engineered** human **cardiac valve** .

Living **tissue - engineered** human **cardiac valve** (50)

pp; 74 DwgNo 18/58

Title Terms: HUMAN; ENGINEERING; TISSUE; TYPE; **HEART** ; **VALVE** ; COMPRISE; ASSEMBLE; LEAFLET; COMPRISE; FIVE; LAYER; LIVE; TISSUE; SHEET; FUSE; FORM ; SELF; SUPPORT; HUMAN; ENGINEERING; TISSUE

Derwent Class: B04; D16; D22; P31; P32

International Patent Class (Main): A61B-000/00; **A61F-002/24** ; A61K-048/00

International Patent Class (Additional): C12N-005/08; C12N-015/02

File Segment: CPI; EngPI

23/5/17 (Item 17 from file: 350)
DIALOG(R)File 350:Derwent WPIX

014815350
WPI Acc No: 2002-636056/200268
Related WPI Acc No: 2000-339483; 2002-589766; 2003-057303; 2003-246572; 2003-255122; 2003-328334; 2003-874531; 2003-874532; 2004-031979; 2004-080009
XRAM Acc No: C04-013457
XRPX Acc No: N04-028864

Semilunar heart valve **useful as prostheses or implants to replace diseased or defective** heart valves , **comprises a biodegradable polymer fiber** scaffold **and** collagen

Patent Assignee: BELL E (BELL-I)
Inventor: BELL E
Number of Countries: 001 Number of Patents: 001
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week
US 20020094573	A1	20020718	US 99420527	A	19991019	200268 B
			US 2000251125	P	20001204	
			US 200124880	A	20011219	

Priority Applications (No Type Date): US 2000251125 P 20001204; US 99420527 A 19991019; US 200124880 A 20011219
Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
US 20020094573	A1		13	A61F-002/24	Div ex application US 99420527 Provisional application US 2000251125

Abstract (Basic): US 20020094573 A1

NOVELTY - A semilunar **heart valve** (I), comprises a biodegradable polymer fiber **scaffold** and **collagen** .

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) making (M1) a semilunar **heart valve** , by assembling a mold which replicates the structure of a semilunar **heart valve** having between two lateral edges a hollow representing the aortic root and hollows representing a number of leaflets with outer and inner surfaces, where the inner surfaces of the hollows represent the number of leaflets connecting with the hollow representing the aortic root and forming the intimal surface of the hollow representing the aortic root, covering the intimal surface of the hollow representing the aortic root and the outside surface of the hollow representing the number of leaflets with a biodegradable polymer fiber **scaffold** , filling the hollow representing the aortic root and the hollows representing the number of leaflets with **collagen** , and freeze-drying the polymer fiber **scaffold** and the **collagen** forming a tissue with two lateral edges;

(2) a semilunar **heart valve** made by the above mentioned method; and

(3) an annular sewing ring (II) for attachment of a **heart valve** to the aortic wall of a host, comprises a biopolymer cloth and a biopolymer rope shaped in a circle, where the biopolymer cloth is wrapped around and stitched to the biopolymer rope.

USE - (M) is useful for making (I) (claimed). (I) or (II) is useful in vitro, for example, for model systems for research, or in vivo as prostheses or implants to replace diseased or defective **heart valves** .

ADVANTAGE - (I) is biocompatible and is processed without the use of crosslinking chemicals such as glutaraldehyde. By eliminating the step of treating (I) with glutaraldehyde or with chemicals similar to glutaraldehyde, the calcification or structural breakdown of the **valve** tissue is eliminated, and binding sites for host human cells and other cells are maintained. (M) preserves the nativity of the biological materials used.

pp; 13 DwgNo 0/4

Title Terms: **HEART** ; **VALVE** ; USEFUL; PROSTHESIS; IMPLANT; REPLACE; DISEASE; DEFECT; **HEART** ; **VALVE** ; COMPRISE; BIODEGRADABLE; POLYMER;

SCAFFOLDING ; COLLAGEN
Derwent Class: B04; D16; D22; P32
International Patent Class (Main): **A61F-002/24**
File Segment: CPI; EngPI

23/5/24 **(Item 24 from file: 350)**
DIALOG(R)File 350:Derwent WPIX

013923669
WPI Acc No: 2001-407882/200143
Related WPI Acc No: 1997-258670; 2002-471182
XRAM Acc No: C01-123467
XRPX Acc No: N01-301809

Hybrid matrices comprising insoluble collagen **fibrils and microcarriers and containing cultured vertebrate cells genetically engineered to express a polypeptide are useful for delivering the peptide e.g. to promote wound healing**

Patent Assignee: TRANSKARYOTIC THERAPIES INC (TRAN-N)
Inventor: ABALOS-COYLE D; LAMSA J C; MINEAU-HANSCHKE R
Number of Countries: 095 Number of Patents: 009
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week	
WO 200124842	A2	20010412	WO 2000US27362	A	20001004	200143	B
AU 200078545	A	20010510	AU 200078545	A	20001004	200143	
BR 200014503	A	20020611	BR 200014503	A	20001004	200248	
			WO 2000US27362	A	20001004		
US 6419920	B1	20020716	US 95548002	A	19951025	200248	
			US 99312246	A	19990514		
			US 99413715	A	19991005		
EP 1221937	A2	20020717	EP 2000968669	A	20001004	200254	
			WO 2000US27362	A	20001004		
CN 1377257	A	20021030	CN 2000813699	A	20001004	200314	
JP 2003511100	W	20030325	WO 2000US27362	A	20001004	200330	
			JP 2001527841	A	20001004		
US 20030091545	A1	20030515	US 95548002	A	19951025	200335	
			US 99312246	A	19990514		
			US 99413715	A	19991005		
			US 2002160452	A	20020531		
MX 2002001450	A1	20020901	WO 2000US27362	A	20001004	200370	
			MX 20021450	A	20020211		

Priority Applications (No Type Date): US 2000662037 A 20000914; US 99413715 A 19991005; US 95548002 A 19951025; US 99312246 A 19990514; US 2002160452 A 20020531
Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
WO 200124842	A2	E	85	A61L-027/00	
Designated States (National): AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW					
Designated States (Regional): AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW					
AU 200078545	A			A61L-027/00	Based on patent WO 200124842
BR 200014503	A			A61L-027/00	Based on patent WO 200124842
US 6419920	B1			A01N-063/00	Div ex application US 95548002 CIP of application US 99312246 Div ex patent US 5965125
EP 1221937	A2	E		A61K-009/00	Based on patent WO 200124842
Designated States (Regional): AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					
CN 1377257	A			A61K-009/00	
JP 2003511100	W		96	A61L-027/00	Based on patent WO 200124842
US 20030091545	A1			A61K-048/00	Div ex application US 95548002 CIP of application US 99312246 Div ex application US 99413715 Div ex patent US 5965125 Div ex patent US 6419920
MX 2002001450	A1			A61K-048/00	Based on patent WO 200124842

Abstract (Basic): WO 200124842 A2
NOVELTY - A composition comprising a body of **matrix** material

(insoluble **collagen** fibrils) containing a population of cultured vertebrate cells genetically engineered to express a polypeptide, a plurality of microcarriers and an agent (a factor which promotes vascularization, a cytokine, a growth factor or ascorbic acid) bound to a solid substrate is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for:

(i) a method of making the composition comprising mixing cultured vertebrate cells genetically engineered to express a polypeptide, a plurality of microcarriers, a solution of soluble **collagen** and an agent bound to substrate, subjecting the soluble **collagen** to conditions to form a gel and exposing the gel to culture conditions which cause the gel to contract to form the body;

(ii) a method of administering a polypeptide comprising implanting the composition containing cultured cells which secrete the polypeptide;

(iii) a method of making the composition comprising mixing cultured vertebrate cells genetically engineered to express a polypeptide, microcarriers and a solution of soluble **collagen** , gelling the mixture, exposing the gel to conditions which cause it to contract and adding keratinocytes;

(iv) a composition comprising a body of **matrix** material comprising insoluble **collagen** fibrils containing a cultured vertebrate cells genetically engineered to express a polypeptide, cultured vertebrate cells secreting a factor which promotes vascularization, a cytokine or a growth factor and microcarriers;

(v) a composition comprising a body of **matrix** material comprising insoluble **collagen** fibrils containing a **cultured fibroblasts** genetically engineered to express a polypeptide, keratinocytes and microcarriers;

(vi) a composition comprising a body of **matrix** material comprising insoluble **collagen** fibrils containing a **cultured fibroblasts** genetically engineered to express a polypeptide, endothelial cells and microcarriers;

(vii) a mixture comprising an aqueous **collagen** solution containing cultured vertebrate cells genetically engineered to express a polypeptide, microcarriers and a factor which promotes vascularization, a cytokine, a growth factor or ascorbic acid bound to a solid substrate;

(viii) a mixture comprising an aqueous **collagen** solution containing cultured vertebrate cells genetically engineered to express a polypeptide, cultured vertebrate cells secreting a factor which promotes vascularization, a cytokine, a growth factor and microspheres;

(ix) a method of administering a polypeptide comprising introducing a fluid mixture comprising an aqueous solution containing cultured vertebrate cells genetically engineered to express a polypeptide and microcarriers;

(x) kits comprising the composition;

(xi) an apparatus for introduction of a mixture into a patient comprising at least two vessels connected to a delivery means.

ACTIVITY - Vulnerary.

MECHANISM OF ACTION - None given.

USE - The composition is useful for administering polypeptides when implanted (especially in a subcutaneous, intraperitoneal, sub-renal capsular, inguinal, intramuscular, intraventricular, intraomental or intrathecal site). The polypeptide preferably promotes wound healing and the composition may be implanted into a pre-existing wound.

ADVANTAGE - The addition of microcarriers to the **collagen matrix** substantially improves the function of the **matrix** .

pp; 85 DwgNo 0/13

Title Terms: HYBRID; **MATRIX** ; COMPRISE; INSOLUBLE; **COLLAGEN** ; FIBRIL; CONTAIN; CULTURE; VERTEBRATE; CELL; GENETIC; ENGINEERING; EXPRESS; POLYPEPTIDE; USEFUL; DELIVER; PEPTIDE; PROMOTE; WOUND; HEAL

Derwent Class: A96; B04; B07; D16; D22; P32; P34

International Patent Class (Main): A01N-063/00; A61K-009/00; A61K-048/00; A61L-027/00

International Patent Class (Additional): **A61F-013/00** ; A61K-031/375; A61K-031/557; A61K-031/5575; A61K-035/12; A61K-038/00; A61K-038/16; A61K-038/21; A61K-038/22; A61K-038/24; A61K-038/27; A61K-038/28;

A61K-038/43; A61K-038/46; A61K-039/00; A61K-047/26; A61K-047/32;
A61K-047/34; A61K-047/36; A61K-047/38; A61K-047/42; A61L-007/54;
A61L-027/24; A61L-027/38; A61L-027/44; A61L-027/54; A61P-017/02;
C07K-014/61; C12N-005/00; C12N-005/08
File Segment: CPI; EngPI

23/5/27 (Item 27 from file: 350)
DIALOG(R)File 350:Derwent WPIX

013587784
WPI Acc No: 2001-071991/200109
XRAM Acc No: C01-020389
XRPX Acc No: N01-054543
In vitro production **of** heart valve **by providing carrier with** fibroblasts **and/or** myofibroblasts **and then** endothelial **cells, followed by treatment in pulsating flow chamber of bioreactor**
Patent Assignee: HOERSTRUP S P (HOER-I); ZUEND G (ZUEN-I); SYMETIS AG (SYME-N)
Inventor: HOERSTRUP S P; ZUEND G
Number of Countries: 025 Number of Patents: 007
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week	
DE 19919625	A1	20001130	DE 199019625	A	19990429	200109	B
EP 1077072	A2	20010221	EP 2000108986	A	20000427	200111	
DE 19919625	C2	20021031	DE 199019625	A	19990429	200273	
EP 1077072	B1	20031112	EP 2000108986	A	20000427	200380	
DE 50004406	G	20031218	DE 4406	A	20000427	200407	
			EP 2000108986	A	20000427		
EP 1077072	B8	20040414	EP 2000108986	A	20000427	200426	
ES 2209715	T3	20040701	EP 2000108986	A	20000427	200444	

Priority Applications (No Type Date): DE 199019625 A 19990429
Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
DE 19919625	A1		12	A61F-002/24	
EP 1077072	A2	G		A61L-027/38	
Designated States (Regional): AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					
DE 19919625	C2			A61F-002/24	
EP 1077072	B1	G		A61L-027/38	
Designated States (Regional): AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					
DE 50004406	G			A61L-027/38	Based on patent EP 1077072
EP 1077072	B8	G		A61L-027/38	
Designated States (Regional): AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					
ES 2209715	T3			A61L-027/38	Based on patent EP 1077072

Abstract (Basic): DE 19919625 A1
NOVELTY - In vitro **production** of a homologous **heart valve** involves:
(i) colonizing a biodegradable carrier with homologous **fibroblasts** and/or **myofibroblasts** so as to give a connective tissue **matrix** ;
(ii) colonizing the **matrix** with **endothelial** cells;
(iii) introducing the **matrix** into a pulsating flow chamber (e.g. in a bioreactor) where it is subjected to a (dis)continuous flow rate.
DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a homologous **heart valve** with a connective tissue inner core covered with a layer of **endothelial** cells, the core having a **collagen** density of 43-55%, based on the **matrix** .
USE - In vitro manufacture of **heart valve** (e.g. **pulmonary** , aortic or **tricuspidal**) prostheses for human implantation.
ADVANTAGE - The prostheses have a connective tissue core surrounded by a surface layer of **endothelial** cells and are thus analogous to natural **valves** ; they have a similar content of **collagen** etc to natural **valves** and can withstand the flow conditions encountered in the human **heart** . Further, they avoid problems associated with prior-art synthetic **valves** such as the need to be supplied with anticoagulants (with the resulting hemorrhaging dangers), a tendency to degenerate and the danger of immunoreaction.
pp; 12 DwgNo 0/5
Title Terms: VITRO; **PRODUCE** ; **HEART** ; **VALVE** ; CARRY; **FIBROBLAST** ; ENDOTHELIUM; CELL; FOLLOW; TREAT; PULSATE; FLOW; CHAMBER

Derwent Class: A23; A96; D16; D22; P32; P34
International Patent Class (Main): **A61F-002/24** ; A61L-027/38
International Patent Class (Additional): A61L-027/00; A61L-033/00; C12N-005/08
File Segment: CPI; EngPI

23/5/28 **(Item 28 from file: 350)**
DIALOG(R)File 350:Derwent WPIX

013565414
WPI Acc No: 2001-049621/200106
XRAM Acc No: C01-013531
XRPX Acc No: N01-038078
Promoting angiogenesis in heart **and vascularization of tissue useful e.g. in** cardiac **surgery using three dimensional stromal tissue**
Patent Assignee: ADVANCED TISSUE SCI INC (ADTI-N); MANSBRIDGE J N (MANS-I); NAUGHTON G K (NAUG-I); PINNEY R E (PINN-I); ZELTINGER J (ZELT-I)
Inventor: MANSBRIDGE J N; NAUGHTON G K; PINNEY R E; ZELTINGER J
Number of Countries: 093 Number of Patents: 005
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week	
WO 200061204	A1	20001019	WO 2000US9848	A	20000412	200106	B
AU 200044564	A	20001114	AU 200044564	A	20000412	200108	
EP 1169069	A1	20020109	EP 2000925950	A	20000412	200205	
			WO 2000US9848	A	20000412		
JP 2002541221	W	20021203	JP 2000610535	A	20000412	200309	
			WO 2000US9848	A	20000412		
US 20030007954	A1	20030109	US 99128838	P	19990412	200311	
			US 99411585	A	19991001		

Priority Applications (No Type Date): US 99411585 A 19991001; US 99128838 P 19990412
Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
WO 200061204	A1	E	61	A61L-027/38	

Designated States (National): AE AG AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
Designated States (Regional): AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW

AU 200044564	A			A61L-027/38	Based on patent WO 200061204
EP 1169069	A1	E		A61L-027/38	Based on patent WO 200061204

Designated States (Regional): AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI

JP 2002541221	W		60	A61K-035/12	Based on patent WO 200061204
US 20030007954	A1			A01N-063/00	Provisional application US 99128838

Abstract (Basic): WO 200061204 A1

NOVELTY - Promoting angiogenesis in the **heart** comprises attaching a three-dimensional stromal tissue to the **heart** to increase the number of blood vessels in the **heart** . The stromal tissue comprises stromal cells and connective tissue proteins naturally secreted by the stromal cells attached to and enveloping a **framework** of a biocompatible, non-living material formed into a three-dimensional structure.

DETAILED DESCRIPTION - Promoting angiogenesis in the **heart** comprises attaching a three-dimensional stromal tissue to the **heart** to increase the number of blood vessels in the **heart** . The stromal tissue comprises stromal cells and connective tissue proteins naturally secreted by the stromal cells attached to and enveloping a **framework** composed of a biocompatible, non-living material formed into a three-dimensional structure having interstitial spaces bridged by the stromal cells.

INDEPENDENT CLAIMS are also included for the following:

(1) a method for promoting healing of an anastomotic site which comprises attaching a three-dimensional stromal tissue (having the above properties) to the site to promote **growth** of **endothelial** cells and increase the number of blood vessels in the site and

(2) a method of promoting vascularization of mammalian tissue in vivo which comprises attaching a three-dimensional stromal tissue to the tissue to increase the number of blood vessels in tissue. The stromal tissue comprises stromal cells attached to and enveloping a **framework** composed of a biocompatible, non-living material formed into

a three-dimensional structure having interstitial spaces bridged by the stromal cells.

USE - Used for promoting blood vessel formation in tissues and organs (especially the **heart**) useful e.g. for promoting repair and regeneration of damaged **cardiac** muscle, promoting vascularization and healing during **cardiac** surgery, promoting blood vessel formation at anastomosis sites and promoting vascularization and repair of damaged skeletal muscle, smooth muscle or connective tissue.

Blood flow at the base of human diabetic foot ulcers treated with three dimensional stromal tissue increased significantly (p is less than 0.001) over 8 weeks treatment, from 325 to 560 arbitrary perfusion units. 5/7 Lesions had healed by week 12 and the other 2 had markedly reduced in size.

pp; 61 DwgNo 0/10

Title Terms: PROMOTE; ANGIOGENESIS; **HEART** ; TISSUE; USEFUL; **CARDIAC** ; SURGICAL; THREE; DIMENSION; STROMA; TISSUE

Derwent Class: A96; B07; D22; P34

International Patent Class (Main): A01N-063/00; A61K-035/12; A61L-027/38

International Patent Class (Additional): A01N-001/00; A01N-065/00; A61K-038/00; A61K-047/12; A61K-047/32; A61K-047/34; A61K-047/38; A61K-047/46; A61L-017/00; A61L-024/00; A61L-027/50; A61L-031/00; A61P-041/00; A61P-043/00

File Segment: CPI; EngPI

23/5/31 (Item 31 from file: 350)
DIALOG(R)File 350:Derwent WPIX

013196004
WPI Acc No: 2000-367877/200032
XRAM Acc No: C00-111253
XRPX Acc No: N00-275360

Artificial skin suitable for wound covering is based on copolymer of polyalkylene glycol and aromatic polyester

Patent Assignee: ISOTIS BV (ISOT-N); ISOTIS NV (ISOT-N)
Inventor: PONEC M; RIESLE J U; VAN BLITTERSWIJK C A; VAN DORP A G M
Number of Countries: 029 Number of Patents: 008
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week
EP 1005873	A1	20000607	EP 99204039	A	19991130	200032 B
AU 9961785	A	20000601	AU 9961785	A	19991130	200035
JP 2000167039	A	20000620	JP 99340836	A	19991130	200036
CA 2291312	A1	20000530	CA 2291312	A	19991130	200043
US 6383220	B1	20020507	US 99451520	A	19991130	200235
US 20020082692	A1	20020627	US 99451520	A	19991130	200245
			US 200124360	A	20011213	
EP 1005873	B1	20030409	EP 99204039	A	19991130	200325
DE 69906668	E	20030515	DE 606668	A	19991130	200340
			EP 99204039	A	19991130	

Priority Applications (No Type Date): EP 98204203 A 19981211; EP 98204031 A 19981130
Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
EP 1005873	A1	E	11	A61L-027/00	
Designated States (Regional): AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					
AU 9961785	A			A61L-027/60	
JP 2000167039	A		10	A61L-027/00	
CA 2291312	A1	E		A61L-027/60	
US 6383220	B1			A61F-002/10	
US 20020082692	A1			A61F-002/10	Cont of application US 99451520
EP 1005873	B1	E		A61L-027/00	
Designated States (Regional): AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					
DE 69906668	E			A61L-027/00	Based on patent EP 1005873

Abstract (Basic): EP 1005873 A1

NOVELTY - Artificial skin based on a copolymer of a polyalkylene glycol and an aromatic polyester has a thickness of 50-2000 microns, and has an upper and lower side, both having a macroporosity of 10-95%.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a method of using an artificial skin in which the skin is applied to a wound of an animal or human.

ACTIVITY - Vulnerary; dermatological. Adhesion was achieved after 1-2 minutes after application for both the cell-free Polyactive (A) and fibroblast-populated Polyactive (B) (**seeded** with autologous or allogeneic fibroblasts). Vascularization using the skin, (A and B respectively) occurred in 4 days (for both) compared to more than 14 days with no treatment. Wound contraction after 30 days after application, was less than 50% (A and B) and more than 50% with no treatment. Neo-dermis formation (comprising **collagen** appearance and distribution, respectively for A and no treatment), was in thin less compact bundles and in parallel arrays compared to B, which was in thick, compact bundles in orthogonal arrays. Degradation (i.e. number of intracellularly located fragments was high for A and low for B.

MECHANISM OF ACTION - No further details.

USE - Used for wound covering or skin replacement in humans and animals (claimed).

ADVANTAGE - The skin adheres well to a wound when applied. The so called 'curling-up' effect which has been found with previous skins does not occur with the present skin. The skin provides a highly

suitable carrier for autologous cells, thus enabling tissue repair. It also forms a highly suitable substrate for culturing epithelial cells, e.g., keratinocytes. Fibroblasts may also be cultured on or within the artificial skin. Due to its porous character, **fibroblasts** will **grow** both on the upper and lower side of the skin. The skin is well tolerated and non-toxic. 24 months post-transplantation, tissue surrounding the Polyactive substrates consisted of a mature connective tissue. At the macroscopic and at the light- and electron-microscopic level, the **heart** , spleen, liver,lung, glands and kidneys did not show any signs of swelling, tissue damage/necrosis or polymer fragments.

pp; 11 DwgNo 0/0

Title Terms: ARTIFICIAL; SKIN; SUIT; WOUND; COVER; BASED; COPOLYMER; GLYCOL ; AROMATIC; POLYESTER

Derwent Class: A23; A96; B07; D16; D22; P32; P34

International Patent Class (Main): **A61F-002/10** ; A61L-027/00; A61L-027/60

International Patent Class (Additional): **A61F-013/00** ; A61L-015/00; C08L-067/02; C08L-071/02

File Segment: CPI; EngPI

23/5/34 **(Item 34 from file: 350)**
DIALOG(R)File 350:Derwent WPIX

012900690
WPI Acc No: 2000-072526/200006
Related WPI Acc No: 2002-739487; 2004-096494
XRAM Acc No: C00-020713
XRPX Acc No: N00-056759
Compositions for regenerating tissue that has degenerated as a result of a disease or disorder
Patent Assignee: ISOLAGEN TECHNOLOGIES INC (ISOL-N)
Inventor: BOSS W K; MARKO O
Number of Countries: 084 Number of Patents: 002
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week	
WO 9960951	A1	19991202	WO 99US11283	A	19990521	200006	B
AU 9940933	A	19991213	AU 9940933	A	19990521	200020	

Priority Applications (No Type Date): US 9883618 A 19980522
Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
WO 9960951	A1	E	60	A61F-002/00	

Designated States (National): AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZA ZW
Designated States (Regional): AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW

AU 9940933	A		A61F-002/00	Based on patent WO 9960951

Abstract (Basic): WO 9960951 A1
NOVELTY - Compositions (I) for promoting regeneration of tissue that has degenerated as a result of a disease or disorder, comprise autologous **fibroblasts** free of immunogenic proteins, optionally integrated within an **acellular matrix** .
DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a method of **producing** (I) comprising incubating a suspension of autologous passaged **fibroblasts** with a biodegradable **acellular matrix** .
ACTIVITY - Regenerative.
MECHANISM OF ACTION - None given.
USE - For promoting regeneration of tissue that has degenerated as a result of diseases or disorders including defects of the oral mucosa, trauma to the oral mucosa (e.g. extraction of a tooth), periodontal disease (e.g. periodontal degeneration, gingivitis, or non-healing wounds of the palatal mucosa or gingival mucosa), diabetes, cutaneous ulcers, venous stasis, scars of the skin or wrinkles of the skin. An injectable composition comprising **collagen** and passaged autologous **fibroblasts** free of immunogenic proteins is used for correcting defects in skin (e.g. wrinkles and scars) and for augmenting tissue, particularly facial tissue.
pp; 60 DwgNo 0/0
Title Terms: COMPOSITION; REGENERATE; TISSUE; DEGENERATE; RESULT; DISEASE; DISORDER
Derwent Class: A96; B04; D16; D21; P32; P34
International Patent Class (Main): **A61F-002/00**
International Patent Class (Additional): **A61F-002/02** ; **A61F-002/10** ; **A61F-013/00** ; A61K-038/00; A61K-038/17; A61M-031/00
File Segment: CPI; EngPI

23/5/35 **(Item 35 from file: 350)**
DIALOG(R)File 350:Derwent WPIX

010443049
WPI Acc No: 1995-344368/199544

XRAM Acc No: C95-151329
XRPX Acc No: N95-257371

Prepn of tissues for implantation - by elimination of native cells and extracellular components and re-population with allogeneic or autologous cells

Patent Assignee: CRYOLIFE INC (CRYO-N)
Inventor: GOLDSTEIN S
Number of Countries: 062 Number of Patents: 012
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week	
WO 9524873	A1	19950921	WO 95US2350	A	19950227	199544	B
AU 9519314	A	19951003	AU 9519314	A	19950227	199602	
US 5613982	A	19970325	US 94213754	A	19940314	199718	
			US 95463455	A	19950605		
US 5632778	A	19970527	US 94213754	A	19940314	199727	
			US 95463643	A	19950605		
JP 9510108	W	19971014	JP 95524033	A	19950227	199751	
			WO 95US2350	A	19950227		
KR 97701523	A	19970412	WO 95US2350	A	19950227	199817	
			KR 96705072	A	19960913		
EP 871414	A1	19981021	EP 95911929	A	19950227	199846	
			WO 95US2350	A	19950227		
US 5843182	A	19981201	US 94213754	A	19940314	199904	
			US 97791450	A	19970127		
US 5899936	A	19990504	US 94213754	A	19940314	199925	
			US 95463171	A	19950605		
EP 871414	B1	20040428	EP 95911929	A	19950227	200429	
			WO 95US2350	A	19950227		
DE 69532976	E	20040603	DE 95632976	A	19950227	200436	
			EP 95911929	A	19950227		
			WO 95US2350	A	19950227		
EP 1452153	A1	20040901	EP 95911929	A	19950227	200457	
			EP 20049948	A	19950227		

Priority Applications (No Type Date): US 94213754 A 19940314; US 95463455 A 19950605; US 95463643 A 19950605; US 97791450 A 19970127; US 95463171 A 19950605
Cited Patents: US 5192312
Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
WO 9524873	A1	E	71	A61F-002/24	
Designated States (National): AM AT AU BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE HU JP KE KG KP KR KZ LK LR LT LU LV MD MG MN MW MX NL NO NZ PL PT RO RU SD SE SI SK TJ TT UA UZ VN					
Designated States (Regional): AT BE CH DE DK ES FR GB GR IE IT KE LU MC MW NL OA PT SD SE SZ UG					
AU 9519314	A			A61F-002/24	Based on patent WO 9524873
US 5613982	A		27	A61F-002/02	Div ex application US 94213754
US 5632778	A		27	A61F-002/02	Div ex application US 94213754
JP 9510108	W		45	C12N-005/06	Based on patent WO 9524873
KR 97701523	A			A61F-002/24	Based on patent WO 9524873
EP 871414	A1	E		A61F-002/24	Based on patent WO 9524873
Designated States (Regional): AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE					
US 5843182	A			A61F-002/24	Cont of application US 94213754
US 5899936	A			A61F-002/24	Div ex application US 94213754
EP 871414	B1	E		A61F-002/24	Based on patent WO 9524873
Designated States (Regional): AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE					
DE 69532976	E			A61F-002/24	Based on patent EP 871414 Based on patent WO 9524873
EP 1452153	A1	E		A61F-002/24	Div ex application EP 95911929 Div ex patent EP 871414
Designated States (Regional): AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE					

Abstract (Basic): WO 9524873 A

The following is claimed:(A) a process for generating implant

tissue which comprises:(a) eliminating native cells and other extracellular tissue components from the tissue to provide a tissue **matrix** ;(b) treating the tissue **matrix** with cellular adhesion factor (CAF) to promote subsequent attachment of **cultured** allogeneic or autologous cells to the surfaces of the tissue **matrix** , and(c) repopulating the tissue **matrix** with the **cultured** allogeneic or autologous cells.

Also claimed are:(B) a process for treating xenogeneic tissue to improve its compatibility with the immune system of an implant recipient of a species different from the species of the source of the native tissue, comprising:(a) applying an amt. of CAFs to a **de - cellularised** tissue **matrix** to promote the subsequent attachment of **cultured** cells to the tissue **matrix** , where the CAF comprises 1 or more extracellular proteins ordinarily associated with the native tissue in a liq. vehicle, and(b) repopulating the tissue **matrix** with autogenous or allogeneic cells to provide a non-immunogenic and bio-mechanically acceptable implant or **graft** which is vitalised by the cellular repopulation and is histologically and biochemically similar to the corresp. natural tissue;(C) a process for generating xenogenic implants or **grafts** from non-human **collagenous** , connective or vascular tissue, where the natural tissue is **de - cellularised** and washed to remove cellular and/or extracellular antigens, followed by treatment of the tissue **matrix** with adhesion factors (AFs) comprised of fibronectin and heparin to promote attachment of **fibroblast** cells immunologically acceptable to the implant or **graft** recipient, where the tissue **matrix** treated with AF is repopulated by incubating the **matrix** in the presence of the **fibroblast** cells and **fibroblast growth** factor (FGF) until such cellular repopulation provides a vitalised tissue histologically similar to the corresp. natural tissue, and where the implant tissue generated is mechanically, biochemically and immunologically suitable for implantation;(D) a process for regenerating a non-immunogenic tissue **matrix** suitable for subsequent processing into implant tissue which comprises eliminating native cells by treating the tissue with components selected from enzymes and nucleases to inhibit subsequent native cell **growth** in the treated tissue and to limit generation of new immunological sites in the tissue;(E) a process for generating a xenogeneic **heart valve** from porcine or bovine **valve** tissue by:(a) **de - cellularising** the native **valve** tissue to provide a **matrix** free of native cellular antigens and treated to limit the generation of new immunological sites, and(b) applying attachment factors to the **valve** tissue **matrix** , comprised of 1 or more extracellular proteins ordinarily associated with the natural tissue, effective to promote attachment of **fibroblast** cells in the presence of **fibroblast growth** factor immunologically acceptable to the implant recipient to provide a vitalised **valve** tissue;(F) a process for generating a **graft** or **heart valve** implant, suitable for use in a human, by treating a porcine **heart** with method (C), and(G) the implant tissue, tissue **matrix** and xenogeneic **valve produced** by the above methods.

USE/ADVANTAGE - The methods are used to treat xenogeneic or allogeneic tissue to generate a viable bio-prosthesis which does not **produce** an adverse immune response by the recipient upon implant, while exhibiting only limited propensity to calcify and little stimulation of thromboembolism. They are used partic. for the prepn. of **heart valves** for implantation (claimed).

Dwg.0/10

Title Terms: PREPARATION; TISSUE; IMPLANT; ELIMINATE; NATIVE; CELL; EXTRACELLULAR; COMPONENT; POPULATION; AUTOLOGOUS; CELL

Derwent Class: B04; D16; D22; P32; P34

International Patent Class (Main): **A61F-002/02** ; **A61F-002/24** ; C12N-005/06

International Patent Class (Additional): A61L-027/00; A61L-027/38; A61L-027/54

File Segment: CPI; EngPI

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Set     Items   Description
S1        178   AU=(HOPKINS R? OR HOPKINS, R?)
S2          2   AU=(HOFFMAN-KIM, D? OR HOFFMAN-KIM D?)
S3          7   AU='HOFFMAN K D'
S4          2   S1 AND (S2 OR S3)
S5          2   (S1 OR S2 OR S3) AND IC=A61F-002?
S6          5   (S1 OR S2 OR S3) AND IC=A61F?
S7          4   (S1 OR S2 OR S3) AND (MYOFIBROBLAST? OR FIBROBLAST? OR END-
             OTHELIAL?)
S8          7   S4 OR S5 OR S6 OR S7
S9          7   IDPAT (sorted in duplicate/non-duplicate order)
S10         7   IDPAT (primary/non-duplicate records only)
File 347:JAPIO Nov 1976-2004/Jun(Updated 041004)
         (c) 2004 JPO & JAPIO
File 348:EUROPEAN PATENTS 1978-2004/Oct W01
         (c) 2004 European Patent Office
File 349:PCT FULLTEXT 1979-2002/UB=20041014,UT=20041007
         (c) 2004 WIPO/Univentio
File 350:Derwent WPIX 1963-2004/UD,UM &UP=200466
         (c) 2004  Thomson Derwent
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10/5/5 **(Item 5 from file: 350)**
DIALOG(R)File 350:Derwent WPIX

014418109 **Image available**
WPI Acc No: 2002-238812/200229
Related WPI Acc No: 2004-419516
XRPX Acc No: N02-184078
Cardiac valve replacement an acellular matrix and isolated myofibroblasts of type I and II collagen to type II
Patent Assignee: HOFFMAN-KIM D (HOFF-I); HOPKINS R A (HOPK-I); RHODE ISLAND HOSPITAL (RHOD-N)
Inventor: **HOFFMAN-KIM D ; HOPKINS R A**
Number of Countries: 001 Number of Patents: 002
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week	
US 20010051824	A1	20011213	US 2000195673	P	20000407	200229	B
			US 2001828768	A	20010409		
US 6652583	B2	20031125	US 2000195673	P	20000407	200378	
			US 2001828768	A	20010409		

Priority Applications (No Type Date): US 2000195673 P 20000407; US 2001828768 A 20010409
Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
US 20010051824	A1		17	A61F-002/24	Provisional application US 2000195673
US 6652583	B2			A61F-002/24	Provisional application US 2000195673

Abstract (Basic): US 20010051824 A1
NOVELTY - The cardiac valve replacement comprises an acellular matrix and isolated **myofibroblasts** , wherein at least 60% of the total collagen produced by the **myofibroblasts** is type I collagen to type II. The **myofibroblasts** produce one or more extracellular matrix components selected from a group consisting of fibronectin, elastin, and glycosaminoglycan.
DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a method of enhancing the production of type I collagen.
USE - For replacing diseased/damaged cardiac/heart valves.
ADVANTAGE - Inhibits dedifferentiation of **myofibroblasts** .
DESCRIPTION OF DRAWING(S) - The drawing shows the cell proliferation of cells derived from a tricuspid valve biopsy.
pp; 17 DwgNo 5/15
Title Terms: CARDIAC; VALVE; REPLACE; ACELLULAR; MATRIX; ISOLATE; TYPE; COLLAGEN; TYPE
Derwent Class: P32
International Patent Class (Main): **A61F-002/24**
File Segment: EngPI

10/5/1 **(Item 1 from file: 350)**
DIALOG(R)File 350:Derwent WPIX

016261622
WPI Acc No: 2004-419516/200439
Related WPI Acc No: 2002-238812
XRAM Acc No: C04-157463
XRPX Acc No: N04-333004
Isolated myofibroblast **for use in bioprosthetic heart valve is genetically altered to increase type I collagen production relative to type III collagen production**
Patent Assignee: HOFFMAN-KIM D (HOFF-I); HOPKINS R A (HOPK-I)
Inventor: **HOFFMAN-KIM D ; HOPKINS R A**
Number of Countries: 001 Number of Patents: 001
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week	
US 20040106991	A1	20040603	US 2000195673	P	20000407	200439	B
			US 2001828768	A	20010409		
			US 2003722279	A	20031124		

Priority Applications (No Type Date): US 2000195673 P 20000407; US 2001828768 A 20010409; US 2003722279 A 20031124
Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
US 20040106991	A1		17	A61F-002/24	Provisional application US 2000195673
					Div ex application US 2001828768
					Div ex patent US 6652583

Abstract (Basic): US 20040106991 A1
NOVELTY - Isolated **myofibroblast** is genetically altered to increase type I collagen production relative to type III collagen production.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:
(1) a bioprosthetic heart valve comprising an acellular matrix and isolated **myofibroblasts** in which at least 60 % of the total collagen produced by the **myofibroblasts** is type I collagen;
(2) enhancing viability and contractile activity of **myofibroblasts** in vitro, which comprises culturing the **myofibroblast** under pulsatile flow conditions;
(3) enhancing production of type I collagen by an isolated **myofibroblast** , which comprises culturing the **myofibroblast** under pulsatile flow conditions; and
(4) manufacturing an artificial heart valve, which comprises providing an acellular matrix, seeding the matrix with isolated **myofibroblasts** , and culturing the **myofibroblasts** under pulsatile flow conditions.
USE - The isolated **myofibroblast** is used in a bioprosthetic heart valve (claimed).
ADVANTAGE - The use of the inventive isolated **myofibroblast** provides a bioprosthetic heart valve eliminates the need for immune suppression when transplanting cells from a donor and eliminates biocompatibility concerns which accompany the use of biomaterials in tissue engineered valves.
pp; 17 DwgNo 0/6
Title Terms: ISOLATE; **MYOFIBROBLAST** ; HEART; VALVE; GENETIC; ALTER; INCREASE; TYPE; COLLAGEN; PRODUCE; RELATIVE; TYPE; COLLAGEN; PRODUCE
Derwent Class: B04; D22; P32
International Patent Class (Main): **A61F-002/24**
File Segment: CPI; EngPI

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Set     Items   Description
S1       1998   AU=(HOPKINS R? OR HOPKINS, R?)
S2         26   AU=(HOFFMAN-KIM, D? OR HOFFMAN-KIM D?)
S3         10   S1 AND S2
S4         42   (S1 OR S2) AND (FIBROBLAST? OR MYOFIBROBLAST? OR ENDOTHELI-
                  AL?)
S5         14   S4 AND (COLLAGEN? OR ACELLULAR? OR DECELLULAR? OR TISSUE()-
                  ENGINEER? OR BIOPROSTHE?)
S6          7   RD (unique items)
File   5:Biosis Previews(R) 1969-2004/Oct W2
         (c) 2004 BIOSIS
File  34:SciSearch(R) Cited Ref Sci 1990-2004/Oct W2
         (c) 2004 Inst for Sci Info
File  35:Dissertation Abs Online 1861-2004/Sep
         (c) 2004 ProQuest Info&Learning
File  65:Inside Conferences 1993-2004/Oct W3
         (c) 2004 BLDSC all rts. reserv.
File 636:Gale Group Newsletter DB(TM) 1987-2004/Oct 20
         (c) 2004 The Gale Group
File  73:EMBASE 1974-2004/Oct W2
         (c) 2004 Elsevier Science B.V.
File  94:JICST-EPlus 1985-2004/Sep W3
         (c)2004 Japan Science and Tech Corp(JST)
File 154:MEDLINE(R) 1990-2004/Oct W3
         (c) format only 2004 The Dialog Corp.
File  98:General Sci Abs/Full-Text 1984-2004/Aug
         (c) 2004 The HW Wilson Co.
File 149:TGG Health&Wellness DB(SM) 1976-2004/Sep W4
         (c) 2004 The Gale Group
File 369:New Scientist 1994-2004/Oct W2
         (c) 2004 Reed Business Information Ltd.
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6/5/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0014642011 BIOSIS NO.: 200400022768
Cardiac valve replacement
AUTHOR: **Hopkins Richard A** (Reprint); **Hoffman-Kim Diane**
AUTHOR ADDRESS: Providence, RI, USA**USA
JOURNAL: Official Gazette of the United States Patent and Trademark Office Patents 1276 (4): Nov. 25, 2003 2003
MEDIUM: e-file
PATENT NUMBER: US 6652583 PATENT DATE GRANTED: November 25, 2003 20031125
PATENT CLASSIFICATION: 623-213 PATENT ASSIGNEE: Rhode Island Hospital
PATENT COUNTRY: USA
ISSN: 0098-1133 (ISSN print)
DOCUMENT TYPE: Patent
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The invention provides a replacement heart valve which contains an **acellular** matrix as a structural scaffold. The scaffold is seeded with isolated **myofibroblasts** and/or **endothelial** cells prior to implantation into a recipient mammal.

DESCRIPTORS:
MAJOR CONCEPTS: Cardiovascular Medicine--Human Medicine, Medical Sciences ; Equipment Apparatus Devices and Instrumentation
BIOSYSTEMATIC NAMES: Mammalia--Vertebrata, Chordata, Animalia
ORGANISMS: mammal (Mammalia)
ORGANISMS: PARTS ETC: **endothelial** cells--circulatory system; **myofibroblast** --muscular system
COMMON TAXONOMIC TERMS: Animals; Chordates; Mammals; Nonhuman Vertebrates ; Nonhuman Mammals; Vertebrates
METHODS & EQUIPMENT: **acellular** matrix--prosthetic; cardiac valve replacement--clinical techniques, therapeutic and prophylactic techniques; replacement heart valve--prosthetic
CONCEPT CODES:
02506 Cytology - Animal
14504 Cardiovascular system - Physiology and biochemistry
14506 Cardiovascular system - Heart pathology
17504 Muscle - Physiology and biochemistry
BIOSYSTEMATIC CODES:
85700 Mammalia

6/5/2 **(Item 2 from file: 5)**
DIALOG(R)File 5:Biosis Previews(R)

0013907670 BIOSIS NO.: 200200501181
Therapeutic potential of implanted tissue - engineered **bioartificial muscles delivering recombinant proteins to the sheep heart**
BOOK TITLE: Annals of the New York Academy of Sciences. Reparative medicine: Growing tissues and organs
AUTHOR: Lu Y; Shansky J; Del Tatto M; Ferland P; McGuire S; Marszalkowski J ; Maish M; **Hopkins R** ; Wang X; Kosnik P; Nackman M; Lee A; Creswick B; Vandenburgh H (Reprint
BOOK AUTHOR/EDITOR: Sipe Jean D; Kelley Christine A; McNicol Lore Anne
AUTHOR ADDRESS: c/o Cell Based Delivery Inc., 4 Richmond Square, Providence, RI, 02906, USA**USA
SERIES TITLE: Annals of the New York Academy of Sciences 961 p78-82 2002
MEDIUM: print
BOOK PUBLISHER: New York Academy of Sciences {a}, 2 East 63rd Street, New York, NY, 10021, USA
CONFERENCE/MEETING: Symposium on Reparative Medicine: Growing Tissues and Organs Bethesda, MD, USA June 25-26, 2001; 20010625
ISSN: 0077-8923 ISBN: 1-57331-382-3 (cloth); 1-57331-383-1 (paper)
DOCUMENT TYPE: Book; Meeting; Book Chapter; Meeting Paper
RECORD TYPE: Citation
LANGUAGE: English
REGISTRY NUMBERS: 67763-96-6: insulin-like growth factor-1; 127464-60-2: vascular **endothelial** growth factor
DESCRIPTORS:
MAJOR CONCEPTS: Cardiovascular System--Transport and Circulation; Genetics; Methods and Techniques; Muscular System--Movement and Support
BIOSYSTEMATIC NAMES: Bovidae--Artiodactyla, Mammalia, Vertebrata, Chordata, Animalia
ORGANISMS: sheep (Bovidae)--adult
ORGANISMS: PARTS ETC: heart--circulatory system; muscle--muscular system, bioartificial, genetic engineering, **tissue - engineered** ; myoblast--muscular system, culture
COMMON TAXONOMIC TERMS: Animals; Artiodactyls; Chordates; Mammals; Nonhuman Vertebrates; Nonhuman Mammals; Vertebrates
DISEASES: ischemic heart disease--heart disease
MESH TERMS: Myocardial Infarction (MeSH)
CHEMICALS & BIOCHEMICALS: insulin-like growth factor-1--secretion; recombinant protein; vascular **endothelial** growth factor--secretion
METHODS & EQUIPMENT: gene therapy--gene therapy method, recombinant gene expression
MISCELLANEOUS TERMS: Book Chapter; Meeting Paper; Meeting Paper
CONCEPT CODES:
00520 General biology - Symposia, transactions and proceedings
02506 Cytology - Animal
03502 Genetics - General
03506 Genetics - Animal
10064 Biochemistry studies - Proteins, peptides and amino acids
14504 Cardiovascular system - Physiology and biochemistry
14506 Cardiovascular system - Heart pathology
17002 Endocrine - General
17504 Muscle - Physiology and biochemistry
BIOSYSTEMATIC CODES:
85715 Bovidae

6/5/3 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0011915832 BIOSIS NO.: 199900175492
Allograft heart valves: The role of apoptosis-mediated cell loss
AUTHOR: Hilbert Stephen L (Reprint); Luna Rafael E; Zhang Jun; Wang Yining; **Hopkins Richard A** ; Yu Zu-Xi; Ferrans Victor J
AUTHOR ADDRESS: Center for Devices and Radiological Health, Food and Drug Administration, 9200 Corporate Blvd, Rockville, MD, 20850, USA**USA
JOURNAL: Journal of Thoracic and Cardiovascular Surgery 117 (3): p454-462 March, 1999 1999
MEDIUM: print
ISSN: 0022-5223
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Objective: The purpose of this study was to determine whether apoptosis of **endothelial** and connective tissue cells is responsible for the loss of cellularity observed in implanted aortic allograft valves. Methods: Fresh (n = 6) and cryopreserved (n = 4) aortic allograft valves were retrieved at 2 days to 20 weeks after implantation in an ovine model. Sections of these valves were studied with the use of histologic and electron microscopic methods, nick end-labeling and dual immunostaining for factor VIII-related antigen and proliferating cell nuclear antigen, followed by counterstaining for DNA and laser scanning confocal fluorescence microscopic observation. Results: The **endothelial** cells and cusp connective tissue cells of implanted valvular allografts showed loss of proliferating cell nuclear antigen (indicative of cessation of mitotic activity) and evidence of apoptosis (nick end labeling). The latter was manifested by nuclear condensation and pyknosis, positive nick end labeling, and formation of intra- and extracellular apoptotic bodies derived from the fragmentation of apoptotic cells. These changes began to develop at 2 days after implantation, peaking at 10 to 14 days, and became complete by 20 weeks, at which time the valves had the typical **acellular** morphologic features of allografts implanted for long periods of time. Conclusions: Apoptosis occurs in **endothelial** cells and cuspal connective tissue cells of implanted allografts and appears to be a cause of their loss of cellularity. This apoptosis may be related to various factors, including immunologic and chemical injury, and hypoxia during valve processing and reperfusion injury at the time of implantation.

DESCRIPTORS:
MAJOR CONCEPTS: Cardiovascular System--Transport and Circulation; Cell Biology; Surgery--Medical Sciences
BIOSYSTEMATIC NAMES: Bovidae--Artiodactyla, Mammalia, Vertebrata, Chordata, Animalia
ORGANISMS: sheep (Bovidae)--breed-Dorset
ORGANISMS: PARTS ETC: cuspal connective tissue cells--skeletal system; **endothelial** cells
COMMON TAXONOMIC TERMS: Animals; Artiodactyls; Chordates; Mammals; Nonhuman Vertebrates; Nonhuman Mammals; Vertebrates
METHODS & EQUIPMENT: allograft heart valve--prosthetic
MISCELLANEOUS TERMS: apoptosis-mediated cell loss
CONCEPT CODES:
14501 Cardiovascular system - General and methods
02506 Cytology - Animal
18001 Bones, joints, fasciae, connective and adipose tissue - General and methods
BIOSYSTEMATIC CODES:
85715 Bovidae

6/5/4 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

13158525 Genuine Article#: 853EG Number of References: 36
Title: Prototype anionic detergent technique used to decellularize **allograft valve conduits evaluated in the right ventricular outflow tract in sheep**
Author(s): Hilbert SL; Yanagida R; Souza J; Wolfinbarger L; Jones AL; Krueger P; Stearns G; Bert A; **Hopkins RA (REPRINT)**
Corporate Source: Rhoe Isl Hosp,Brown Med Sch, Div Cardiac Surg,2 Dudley St,500/Providence//RI/02905 (REPRINT); US FDA,Ctr Devices & Radiol Hlth , Off Sci & Engn Labs,Rockville//MD/20857; Rhode Isl Hosp,Collis Cardiac Surg Res Lab,Providence//RI/02903; Brown Med Sch,Div Cardiothorac Surg, Dept Surg,Providence//RI/; LifeNet,Virginia Beach//VA/(RAHopkins@lifespan.org)
Journal: JOURNAL OF HEART VALVE DISEASE, 2004, V13, N5 (SEP), P831-840
ISSN: 0966-8519 Publication date: 20040900
Publisher: I C R PUBLISHERS, CRISPIN HOUSE, 12/A SOUTH APPROACH, MOOR PARK, NORTHWOOD HA6 2ET, ENGLAND
Language: English Document Type: ARTICLE
Geographic Location: USA
Journal Subject Category: CARDIAC & CARDIOVASCULAR SYSTEMS
Abstract: Background and aim of the study: Biodegradable polymeric materials or extracellular matrix scaffolds are used in **tissue - engineered** heart valve designs, with the expectation of replicating the anatomic, histological and biomechanical characteristics of semilunar valves. The study aim was to evaluate the extent of in-vivo recellularization and the explant pathology findings of a prototype anionic, non-denaturing detergent and endonuclease technique used to **decellularize** allograft (homograft) valve conduits implanted in the right ventricular outflow tract (RVOT) of sheep, and to identify possible risks associated with **tissue - engineered** heart valve conduits based on **decellularized** allograft semilunar valve scaffolds.

Methods: Valve conduits were **decellularized** using a solution of N-lauroylsarcosinate and endonucleases, rinsed in lactated Ringers solution, and stored in an antibiotic solution at 4degreesC until implanted. Explanted valves and unimplanted controls were examined macroscopically, radiographically (for calcification) and histologically using immunohistochemistry (IHC), routine and special histological stains, transmission electron microscopy (TEM) and polarized light microscopy (evaluation of **collagen** crimp).

Results: Cells and cellular remnants were uniformly absent in the **decellularized** cusps, but occasional focal sites of arterial wall smooth muscle cells and to a greater extent subvalvular cardiac myocytes were variably retained. The trilaminar histological structure of the cusp was preserved. Valve conduit-related pathology consisted of intracuspal hematoma formation, **collagen** fraying, thinning of the conduit wall, and inflammatory cells associated with cardiac myocyte remnants. Cuspal calcification was not seen, but elastic fibers in the conduit wall and retained subvalvular cardiac myocyte remnants were liable to calcification. Fibrous sheath formation was present on the luminal surface of the conduit and extended over the cuspal surfaces to a variable extent. **Myofibroblast** -like cells repopulated the conduit wall and the basal region of the cusp. Re- **endothelialization** was variably present on the cuspal surfaces.

Conclusion: Explant pathology findings showed that in-vivo recellularization occurred, but was focally limited to regions of the arterial wall and cusp base. Safety concerns related to detergent and endonuclease use were identified. Methods to eliminate the potential for structural deterioration and enhance the rate and extent of recellularization of valve conduit tissue are required. Pathology findings showed implantation of valve conduits in the RVOT of juvenile sheep for 20 weeks to be a reliable animal model for the initial

in-vivo assessment of **decellularized** valves. A 20-week period may be insufficient however to evaluate the long-term safety and effectiveness of a **tissue - engineered** valve conduit, as these depend on effective and phenotypically appropriate recellularization accompanied by sustained cell viability and function.

Identifiers--KeyWord Plus(R): ENGINEERED HEART-VALVES; CARDIAC VALVES; IN-VITRO; TISSUE; REPLACEMENT; **MYOFIBROBLAST**; RESTORATION; EXPERIENCE; HOMOGRAFTS; LEAFLETS

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MESSIER RH, 1994, V57, P1, J SURG RES
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6/5/5 **(Item 2 from file: 34)**
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

11493677 Genuine Article#: 659AA Number of References: 19
Title: Tricuspid valve biopsy: A potential source of cardiac myofibroblast **cells for** tissue - engineered **cardiac valves**
Author(s): Maish MS; **Hoffman-Kim D** ; Krueger PM; Souza JM; Harper JJ; **Hopkins RA (REPRINT)**
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Journal: JOURNAL OF HEART VALVE DISEASE, 2003, V12, N2 (MAR), P264-269
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Journal Subject Category: CARDIAC & CARDIOVASCULAR SYSTEMS
Abstract: Background and aims of the study: As progress is made in the development of a **tissue - engineered** cardiac valve, the need for a reliable cell source is particularly important. A technique has been developed for the reliable biopsy of tricuspid valve leaflets. Expanding the harvested cells in culture is feasible and provides a source of leaflet cells that are structurally and functionally similar to the pulmonary and aortic valve leaflet cells that they may replace.

Methods: Thirteen sheep underwent tricuspid valve biopsy. Transthoracic echocardiography (TTE) was performed to evaluate function and guide the subsequent biopsy. **Myofibroblasts** were isolated from the biopsy samples, expanded in culture through 10 passages, and evaluated with immunocytochemistry for valve cell markers. Two animals were sacrificed acutely, two animals died during the immediate postoperative period, and nine animals survived for four weeks or more.

Results: All preoperative and pre-explantation echocardiograms were normal. Both animals sacrificed acutely showed that the tricuspid valve leaflet was indeed biopsied with this technique. Two perioperative deaths occurred; one animal died secondary to injury of the chorda tendinea with subsequent destruction of the posterior leaflet; another died from disruption of the superior vena cava that led to irreversible cardiac tamponade. At sacrifice 2 to 17 weeks), all other animals showed intact tricuspid valves with normal leaflet anatomy. All cultured biopsies generated **myofibroblasts** that, were immunocytochemically positive for alpha smooth muscle actin, chondroitin sulfate, vimentin and fibronectin.

Conclusion: Biopsy of the tricuspid valve to obtain recipient cardiac valve leaflet cells is possible, and the technique is simple and reliable. Biopsy of the leaflet does not compromise function. Interstitial cells can be harvested and expanded in culture. Cellular structure and function is preserved and is similar to that of other cardiac leaflet cells. Tricuspid valve leaflet biopsies are a potential source for harvesting cells to be used in the development of a **tissue - engineered** cardiac valve.
Identifiers--KeyWord Plus(R): **BIOPROSTHETIC** HEART-VALVE; INTERSTITIAL-CELLS; IN-VITRO; EXTRACELLULAR-MATRIX
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6/5/6 **(Item 1 from file: 73)**
DIALOG(R)File 73:EMBASE

10554238 EMBASE No: 2000019516
Tissue engineering **of autologous aorta using a new biodegradable polymer**
Shum-Tim D.; Stock U.; Hrkach J.; Shinoka T.; Lien J.; Moses M.A.; Stamp A.; Taylor G.; Moran A.M.; Landis W.; Langer R.; Vacanti J.P.; Mayer J.E. Jr.; **Hopkins R.A.**
Dr. J.E. Mayer Jr., Department of Cardiovascular Surgery, Children's Hospital, 300 Longwood Ave, Boston, MA 02115 United States
Annals of Thoracic Surgery (ANN. THORAC. SURG.) (United States) 1999, 68/6 (2298-2305)
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Background. Ovine pulmonary valve leaflets and pulmonary arteries have been **tissue - engineered** (TE) from autologous cells and biodegradable polyglycolic acid (PGA)-polyglactin copolymers. Use of this cell-polymer construct in the systemic circulation resulted in aneurysm formation. This study evaluates a TE vascular graft in the systemic circulation which is based on a new copolymer of PGA and polyhydroxyalkanoate (PHA). Methods. Ovine carotid arteries were harvested, expanded in vitro, and seeded onto 7- mm diameter PHA-PGA tubular scaffolds. The autologous cell-polymer vascular constructs were used to replace 3-4 cm abdominal aortic segments in lambs (group TE, n = 7). In a control group (n = 4), aortic segments were replaced with **acellular** polymer tubes. Vascular patency was evaluated with echography. All control animals were sacrificed when the grafts became occluded. Animals in TE group were sacrificed at 10 days (n = 1), 3 (n = 3), and 5 months (n = 3). Explanted TE conduits were evaluated for **collagen** content, deoxyribonucleic acid (DNA) content, structural and ultrastructural examination, mechanical strength, and matrix metalloproteinase (MMP) activity. Results. The 4 control conduits became occluded at 1, 2, 55, and 101 days. All TE grafts remained patent, and no aneurysms developed by the time of sacrifice. There was one mild stenosis at the anastomotic site after 5 months postoperatively. The percent **collagen** and DNA contents approached the native aorta over time (% **collagen** = 25.7% +/- 3.4 [3 months] vs 99.6% +/- 11.7 [5 months], $p < 0.05$; and % DNA = 30.8% +/- 6.0 [3 months] vs 150.5% +/- 16.9 [5 months], $p < 0.05$). Histology demonstrated elastic fibers in the medial layer and **endothelial** specific von Willebrand factor on the luminal surface. The mechanical strain-stress curve of the TE aorta approached that of the native vessel. A 66 kDa MMP-2 was found in the TE and native aorta but not in control group. Conclusions. Autologous aortic grafts with biological characteristics resembling the native aorta can be created using TE approach. This may allow the development of 'live' vascular grafts.

DRUG DESCRIPTORS:
*polymer
MEDICAL DESCRIPTORS:
*bioengineering; *aorta graft
pulmonary valve; systemic circulation; carotid artery; histology; cell isolation; explant; nonhuman; animal experiment; controlled study; animal tissue; article; priority journal
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018 Cardiovascular Diseases and Cardiovascular Surgery
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Suppression of type I collagen gene expression by prostaglandins in fibroblasts is mediated at the transcriptional level.

Riquet FB, Lai WF, Birkhead JR, Suen LF, Karsenty G, Goldring MB.

Rheumatology Division, Beth Israel Deaconess Medical Center, New England Baptist Bone & Joint Institute, Harvard Institutes of Medicine, Boston, Massachusetts, USA.

BACKGROUND: Tissues undergoing a chronic inflammatory process, such as the synovium in rheumatoid arthritis, are characterized by the infiltration of lymphocytes of different subsets and activation of monocyte/macrophages. Interleukin-1 (IL-1), a monocyte/ macrophage product that stimulates synovial fibroblasts to produce matrix metalloproteinases (MMPs), prostaglandins, and other cytokines, also has profound effects on the synthesis of extracellular matrix components such as type I collagen. In previous studies, we have shown that synovial fibroblasts and chondrocytes isolated from human joint tissues are particularly sensitive to prostaglandins, which modulate the effects of IL-1 on collagen gene expression in an autocrine manner. MATERIALS AND METHODS: BALBc/3T3 fibroblasts were treated with IL-1 and prostaglandins in the absence and presence of indomethacin to inhibit endogenous prostaglandin biosynthesis. Collagen synthesis was analyzed by SDS-PAGE as [3H]proline-labeled, secreted proteins, and prostaglandin production and cyclic adenosine 3',5'-cyclic monophosphate (camp) content were assayed. The expression of type I collagen gene (Col1a1) promoter-reporter gene constructs was examined in transient transfection experiments, and the binding of nuclear factors to the Col1a1 promoter region spanning -222 bp/+ 116 bp was analyzed by DNase I footprinting and electrophoretic mobility shift (EMSA) assays. RESULTS: IL-1 increased the synthesis of type I and type III collagens in BALBc/3T3 fibroblasts; greater increases were observed when IL-1-stimulated synthesis of PGE2 was blocked by indomethacin. Transient transfection experiments demonstrated dose-dependent inhibition of the-222 bp Col1a1 promoter by exogenously added prostaglandins with the order of potency of PGF2alpha > PGE2 > PGE1 DNase I footprinting showed increased protection, which extended from the region immediately upstream of the TATA box, owing to the binding of nuclear factors from PGE2- or PGE1-treated BALBc/3T3 cells. EMSA analysis showed zinc-dependent differences in the binding of nuclear factors from untreated and prostaglandin-treated cells to the -84 bp/-29 bp region of the Col1a1 promoter. CONCLUSIONS: These results show that the inhibition of Col1a1 expression by IL-1 in fibroblasts is mediated by prostaglandins at the transcriptional level and suggest that PGE-responsive factors may interact directly or indirectly with basal regulatory elements in the proximal promoter region of the Col1a1 gene.

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Differences in the mechanism for high- versus moderate-density fibroblast-populated collagen lattice contraction.

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Section of Plastic and Reconstructive Surgery, Milton S. Hershey Medical Center, Hershey, Pennsylvania, USA. pehrlicsu.edu

The free-floating fibroblast-populated collagen lattice (FPCL) model introduced by Bell contains 0.5 x 10(5) cell/ml and here is defined as a moderate-density FPCL (MD-FPCL). One modification of the model is to increase the cell density by a factor of 10, where 5 x 10(5) cells/ml defines a high-density FPCL (HD-FPCL). The initial detection of HD-FPCL contraction is 2 h, whereas MD-FPCL is later, 6 h. A contracted HD-FPCL has a doughnut-like appearance, due to the high density of cells accumulating at the periphery. A contracted MD-FPCL is a flattened disc. The compacted collagen of MD-FPCL lattice exhibits a strong birefringence pattern due to organized collagen fiber bundles. In contracted HD-FPCL, a minimal birefringence develops, indicating minimal organization of collagen fiber bundles. MD-FPCL contraction was reduced with less than 10% serum; the disruption of microtubules, uncoupling of gap junctions, inhibition of tyrosine kinases, and addition of a blocking antibody to alpha2beta1 collagen integrin. Making HD-FPCL with only 1% serum or including the inhibitory agents had only minimal affect on lattice contraction. On the other hand, platelet-derived growth factor stimulated HD-FPCL contraction but had no influence on MD-FPCL contraction. It is suggested that the mechanism for HD-FPCL contraction is limited to the process of cells spreading. HD-FPCL contraction is independent of collagen organization, microtubules, gap junctions, alpha2beta1 integrin, and tyrosine phosphorylation. MD-FPCL contraction involves collagen organization and is optimized by the involvement of microtubules, gap junctions, alpha2beta1 integrin, and tyrosine phosphorylation. When studying cell physiology in a collagen matrix, cell-density influences need to be considered. 2000 Wiley-Liss, Inc.

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ELSEVIER SCIENCE FULL-TEXT ARTICLE

Tissue engineering of heart valves--human endothelial cell seedin detergent acellularized porcine valves.

Bader A, Schilling T, Teebken OE, Brandes G, Herden T, Steinhoff G, Haverich A.

Leibniz Research Laboratories for Biotechnology and Artificial Organs, LEB Hannover Medical School, Germany. abader@artificial-organs.de

OBJECTIVE: Tissue engineering of heart valves represents a new experimen concept to improve current modes of therapy in valvular heart disease. Drawb of glutaraldehyde fixed tissue valves or mechanical valves include the short durability or the need for life-long anticoagulation, respectively. Both have in common the inability to grow, which makes valvular heart disease especially problematic in children. The aim of this study was to develop a new methodo for a tissue engineered heart valve combining human cells and a xenogenic acellularized matrix. METHODS: Porcine aortic valves were acellularized by deterging cell extraction using Triton without tanning. Endothelial cells were isolated in parallel from human saphenous veins and expanded in vitro. Speci of the surface of the acellular matrix were seeded with endothelial cells. Anal of acellularity was performed by light microscopy and scanning electron microscopy. Cell viability following seeding was assayed by fluorescence sta of viable cells. RESULTS: The acellularization procedure resulted in an almo complete removal of the original cells while the 3D matrix was loosened at interfibrillar zones. However the 3D arrangement of the matrix fibers was gro maintained. The porcine matrix could be seeded with in vitro expanded huma endothelial cells and was maintained in culture for up to 3 days to document formation of confluent cultures. CONCLUSIONS: Porcine aortic valves can almost completely acellularized by a non-tanning detergent extraction proced The xenogenic matrix was reseeded with human endothelial cells. This appro may eventually lead to the engineering of tissue heart valves repopulated with patients own autologous cells.

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LIVING ARTIFICIAL HEART VALVE ALTERNATIVES: A REVIEW

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Abstract

Conventional replacement therapies for heart valve disease are associated with significant drawbacks. The field of tissue engineering has emerged as an exciting alternative in the search for improved heart valve replacement structures. One of the principles behind this concept is the transplantation of living elements, embedded in a suitable scaffold material, to the diseased site where the structure becomes integrated with patients' tissue to restore natural function. Significant progress has been made in the last ten years in the development of a living artificial heart valve alternative (LAHVA), with the identification of potential replacement sources for valve cells, scaffolds to maintain the cells in a three-dimensional environment, and signals to promote tissue development. This review addresses the need for a tissue-engineered alternative to current prostheses and provides a detailed account of normal heart valve structure – the blueprint for LAHVA fabrication. The research efforts to create a viable LAHVA, including recent developments, are discussed. Particular attention is focused on the choice of cell source for LAHVA construction, the use of biodegradable natural and synthetic polymeric scaffolds as extracellular matrix derivatives, and exogenous stimulation of tissue growth. The critical challenges involved in LAHVA development and possible future areas of investigation are also discussed.

Key Words: Heart valve, tissue engineering, cardiovascular system, extracellular matrix, heart valve prosthesis, interstitial cell, endocardial cell, biodegradable scaffold, bioreactor, growth factor.

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Introduction

Heart valve disease has a devastating impact worldwide; the American Heart Association reports an estimated 87,000 heart valve replacement procedures in 2000 (American Heart Association, 2002), while approximately 275,000 procedures are performed globally each year (Rabkin and Schoen, 2002). Cited as the cause of death in 19,737 cases during 2000 in the US alone, heart valve disease was a contributing factor in an estimated 42,300 further cases (American Heart Association, 2002). Heart valve disease occurs when one or more of the four heart valves can no longer perform their function adequately as gateways in the circulation, failing to maintain a competent unidirectional flow of blood through the heart. Two principal types of valve disease can develop which prevent the valves from opening or closing properly. The first type, valvar stenosis, is characterised by a marked narrowing of the valve opening. The second type, valvar insufficiency, occurs when the valve does not form a tight seal upon closure, resulting in regurgitation of blood. Both disease types burden the heart with an increased work rate to maintain stroke volume, leading to heart muscle dysfunction and eventually heart failure (Carabello and Crawford, 1997). Although the treatment of choice for many years has been surgical valve repair (Alvarez and Murday, 1995), complete valve replacement is warranted in the most advanced of cases.

Current Treatment and Associated Limitations

Exploration into heart valve replacement began in the 1950s, with the first successful human valve implantation being performed in 1952 (Hufnagel *et al.*, 1952). Subsequent decades saw the development of more than 80 designs of prosthetic heart valves (Vongpatanasin *et al.*, 1996); these remain the most common treatment for advanced heart valve disease (Schoen and Levy, 1999). Prosthetic heart valves may be either mechanical, consisting entirely of synthetic components, or may be fashioned from biological tissue (bioprosthetic). 55% of implanted valves worldwide are mechanical, with the remaining 45% bioprosthetic (Butany *et al.*, 2003a); while both types prolong life as well as enhancing its quality, they are associated with a number of major complications that limit their success (Schoen and Levy, 1994).

Advances in engineering and biomaterials have enabled the design of efficient mechanical heart valves (Korossis *et al.*, 2000). The major advantage of mechanical heart valves is their durability and longevity (life span > 25 years) – making them more suitable than bioprosthetic valves for

paediatric patients. However, the body's natural response to foreign materials can often result in thrombosis, which can lead to mortality. For this reason, patients receiving mechanical heart valves are placed on long-term anticoagulation therapy. Other limitations with mechanical valves are their association with infective endocarditis, risk of failure or separation of valvar components which may become embolised (Vongpatanasin *et al.*, 1996).

Bioprosthetic valves may be constructed from either animal-derived tissue (xenografts) or human-derived tissue (homografts) (Butany *et al.*, 2003b). Xenografts are typically composed of either porcine aortic tissue or bovine pericardial tissue, and are normally mounted on a supporting frame. These valves are generally treated with a chemical cross-linking agent, such as glutaraldehyde, in order to increase their durability. Advantages of xenografts include an unlimited supply of donor tissue and superior hemodynamics to mechanical valves (Butany *et al.*, 2003b). Additionally, recipients generally do not require anticoagulation treatment since the surface of bioprosthetic valves carries a lower thrombogenic risk than mechanical valves. However, progressive tissue deterioration afflicts biological replacements with a significantly short life-span (10-15 years) (Hammermeister *et al.*, 1993), and consequently they are seldom used in younger patients in whom they are more susceptible to calcification (Curcio *et al.*, 1981; al-Khaja *et al.*, 1991).

Cryopreserved cadaveric homografts or healthy valves from heart transplant patients are also used as valve replacements, the natural biological form of the leaflets contributing to the enhanced functional performance of the graft. The tissue contains viable, living cells, making it more biocompatible than either mechanical or xenograft valves. Thromboembolic complications are also quite low (Mitchell *et al.*, 1995). In spite of these advantages, however, the long-term function and durability of homografts is still inadequate (Angell *et al.*, 1989). There is always a risk of damage to the valves through chronic rejection, with the foreign tissue evoking an immune response in the patient. Although there is little evidence of acute rejection, there are reports of rapid failure of homograft valves in infants (Rajani *et al.*, 1998). A further drawback of homografts lies in their limited supply due to a shortage of donor tissue.

The underlying problem with all current methods of heart valve replacement is that the non-living material lacks an intrinsic repair system that can recondition the valve after structural injury. Optimal valve replacement tissue should be biocompatible whilst exhibiting growth and reparative capabilities (Nugent and Edelman, 2003). In recent years investigators have begun to focus their efforts on the development of living, functional replacements for tissue damaged through disease or injury (Langer and Vacanti, 1993). Current strategies in this arena of tissue engineering include transplantation of constructs containing specific cell types to the site of injury following an *in vitro* conditioning period (*in vitro* colonisation approach), or constructs which will recruit endogenous progenitor or differentiated cells from the surrounding tissue (*in vivo* colonisation approach). Either of these constructs, theoretically, could functionally integrate with the host tissue and encourage tissue regeneration. A thorough knowledge of normal heart valve development and functional tissue composition is an essential prerequisite to establish the desired structural make-up of a tissue-engineered alternative (Rabkin and Schoen, 2002).

Heart Valve Structure – The Blueprint for a Tissue-Engineered Replacement

The four heart valves arise from embryonic mesenchymal outgrowths referred to as the endocardial cushions in a process termed valvuloseptal morphogenesis (Eisenberg and Markwald, 1995); two valves form in the atrioventicular (AV) canal (the tricuspid (trileaflet) and mitral (bileaflet) AV valves), with two valves forming in the ventricular outflow tract (aortic and pulmonary arterial valves – both trileaflet). It has been widely accepted that all heart valves are simple structures whose contribution to the unidirectional flow of blood was merely passive movement in response to pressure gradients across the valve (Cooper *et al.*, 1966). This understanding of 'passive structures' led to the development of the 'passive' mechanical and bioprosthetic valve replacements. It is now becoming apparent, however, that the heart valves have a more complex structure specialised for the microenvironment in which they are placed (Yacoub *et al.*, 1999). A living artificial heart valve alternative (LAHVA) will need to assume an identical structure if it is to replace the current modes of valve treatment; an in-depth knowledge of valvar components and their interaction will be critical to accomplish significant breakthroughs in the field.

Principal heart valve cell types

The principal cell types in the heart valve are the valvar interstitial cells (VICs) and valvar endocardial cells (VECs), with smooth muscle, cardiac muscle and nerve cells also present to varying degrees depending on the specific valve under consideration (Cooper *et al.*, 1966; Fenoglio *et al.*, 1972; Hibbs and Ellison, 1973).

Valvar interstitial cells. VICs, believed to be responsible for maintenance of valvar structure, are numerous, elongated cells with many long, slender processes extending throughout the valve matrix (Filip *et al.*, 1986). They connect to each other to establish a three-dimensional network throughout the entire valve, and are intimately associated with the valve matrix. It has been suggested that there may be two morphologically and structurally distinct populations of VICs – one possessing contractile properties, characterised by prominent stress fibres, and one possessing secretory properties, characterised by prominent rough endoplasmic reticulum (rER) and Golgi apparatus (Filip *et al.*, 1986; Lester *et al.*, 1988; Zacks *et al.*, 1991). Components characteristic of smooth muscle cells suggest VICs have functional capabilities other than fibroblast matrix secretion (Filip *et al.*, 1986; Messier *et al.*, 1994), and it is possible that *in vivo* some VICs are able to contract in order to maintain a limited intrinsic valvar force and withstand hemodynamic pressures (Mulholland and Gotlieb, 1997). Contractile function is further supported by the expression of both cardiac and skeletal contractile

proteins in VICs, which include α- and β-myosin heavy chain and various troponin isoforms (Roy *et al.*, 2000). Valve leaflet contraction has been demonstrated in response to a range of vasoactive agents, suggesting a coordinated contribution of biological stimuli to successful valve function (Chester *et* al., 2000; Chester *et* al., 2001; Misfeld *et al.*, 2002). The main characteristics of VICs are summarised in Table 1.

VICs are also the essential components of the intrinsic repair system of the valve. The constant motion of the valve leaflets, and connective tissue deformations associated with that movement produce damage to which the VICs respond in order to maintain valvar integrity. This regenerative process appears to be vital to normal valvar function (Schneider and Deck, 1981; Henney *et al.*, 1982), and the absence of VICs in current prosthetic heart valve models is a probable factor contributing to structural failure.

Although extensive data exists regarding the heterogeneity and reparative properties of the VIC population, their function is still quite vague. An important avenue of VIC research will be translation of the cross-talk between the cells and their surrounding matrix mediated through focal adhesion molecules. Focal adhesions are specialised cell-matrix attachment sites linking the cell cytoskeleton to the matrix proteins via integrins. They also act as signal transduction sites, transmitting mechanical information from the ECM, which can elicit a number of responses including, but not limited to, cell adhesion, migration, growth and differentiation (Sastry and Burridge, 2000; Weber *et al.*, 2002). An understanding of the cell biology of VICs is vital in order to determine the mechanisms by which these cells interact with each other and their surrounding environment, in order that this function can be reproduced in a LAHVA.

Valvar endocardial cells. The other major valve cell type is the VEC, which forms a functional envelope around each of the four heart valves. Whilst there is significant data on the various functions of vascular endothelium (Bachetti and Morbidelli, 2000), the specific role of valve endocardium remains unclear and has not been widely studied. However, it seems reasonable that the VECs act to maintain a non-thrombogenic valve surface, similar to the vascular endothelium (Frater *et al.*, 1992). Current valve replacements are devoid of a functional, protective endocardium. This may result in platelet and fibrin deposits on glutaraldehyde-fixed bioprosthetic valves, bacterial infection and tissue calcification (Frater *et al.*, 1992; Lehner *et al.*, 1997).

Another likely function of VECs is the regulation of the underlying VICs, similar to the regulation of vascular smooth muscle cells by the endothelium (Mulholland and Gotlieb, 1997). A complex interaction exists between endothelium and neighbouring cells, mediated in part by soluble factors secreted by endothelial cells (Guarda *et al.*, 1993). A sensory role for VECs has also been proposed by Hill and Folan-Curran (1993); these cells present a large surface area covered with microappendages on their luminal aspect thus increasing exposure to, and possible interaction with, metabolic substances in the circulating blood.

Endothelium often displays morphological and functional differences related to the stress generated by blood flow, and the same is true for VECs, with both elongated and polygonal cells being observed (Hurle *et al.*, 1985). The changes in cell structure may be due to the effects of local hemodynamics on either the cytoskeletal components of the cell, or a secondary effect incurred by changes in the underlying extracellular matrix material. Ultrastructurally, VECs have been shown to possess cell junctions, plasmalemmal vesicles and rER (Table 2); although they express von Willebrand factor (vWf) both *in vitro* and *in vivo*, they lack Weibel-Palade bodies {specific storage granules for von Willebrand factor} (Lester *et al.*, 1993), which are characteristic organelles of vascular endothelium. *In situ*, abutting VECs are linked together by tight junctions and gap junctions as well as overlapping marginal edges – the so-called marginal folds (Harasaki *et al.*, 1975; Manduteanu *et al.*, 1988).

Endothelial cells and endocardial cells preserve some of their main metabolic activities *in vitro* (Manduteanu *et al.*, 1988), providing suitable cell markers for their identification. The cells express vWf in two-dimensional cell culture (Manduteanu *et al.*, 1988) and demonstrate angiotensin converting enzyme (ACE) activity (Manduteanu *et al.*, 1988; Bachetti and Morbidelli, 2000). The cells have also been shown to produce prostacyclin (Manduteanu *et al.*, 1988) and have demonstrated endothelial nitric oxide synthase (eNOS) expression and activity (Smith *et* al., 1993; Siney and Lewis, 1993). These markers are useful in identifying ideal cell culture isolates in developing a LAHVA, but the immune-stimulating potential of VECs themselves may limit their use in tissue-engineered structures. Simon *et al.* (1993) report the expression of major histocompatability complex (MHC) antigens on cultured VECs, suggesting that an allogeneic valve may provoke immunological reactions in the recipient contributing to rapid valve degeneration. VECs also express the adhesion molecules ICAM-1 and ELAM-1, which are important for the binding of mononuclear cells during an immune response (Simon *et al.*, 1993). Batten *et al.* (2001) have demonstrated a similar range of MHC and adhesion/co-stimulatory molecules in VECs and VICs, but only VECs were immunogenic. Although patient-derived autologous VECs present an ideal cell source to construct a LAHVA it may not be clinically feasible to harvest an adequate valve tissue sample. Finding a suitable endocardial replacement cell source is just one of the many hurdles that must be overcome in realising the goal of a LAHVA.

Heart valve extracellular matrix

Histologically, the heart valve leaflet consists of a valve interstitial matrix enveloped by a continuous monolayer of VECs. The principal components of the valve extracellular matrix (ECM) are the fibrous macromolecules collagen and elastin, proteoglycans and glycoproteins, each component conferring unique physical and mechanical properties (Kunzelman *et al.*, 1993). Collagen accounts for ~60% dry weight of the valve, elastin for ~10% and proteoglycans for ~20% (Kunzelman *et al.*, 1993). The collagen component, which provides most of the mechanical strength of the valve, is predominantly type I and III (74% and 24% respectively) with ~2% of type V collagen (Cole *et al.*, 1984). The collagen

Table 1 Characteristics of valvar interstitial cells (VICs)

Anatomical or Physiological Feature:	**Evidenced by:**	**Reference:**
Cytoskeleton	α-smooth muscle actin (α-SMA) staining	Filip *et al.*, 1986; Taylor *et al.*, 2000
	Vimentin staining	Lester *et al.*, 1988; Messier *et al.*, 1994; Taylor *et al.*, 2000
	Desmin staining	Messier *et al.*, 1994; Taylor *et al.*, 2000
	Smooth muscle cell myosin staining	Lester *et al.*, 1988; Taylor *et al.*, 2000
	Troponin T, I, C staining	Roy *et al.*, 2000
	α-myosin heavy chain (α-MHC) staining	Roy *et al.*, 2000
	β-myosin heavy chain (β-MHC) staining	Roy *et al.*, 2000
	Cardiac myosin light chain-2 (MLC2) staining	Roy *et al.*, 2000
	α-tubulin staining	Messier *et al.*, 1994
	β-tubulin staining	Taylor *et al.*, 2000
Contractility	Positive response to epinephrine, angiotensin II	Filip *et al.*, 1986, Messier *et al.*, 1994
	Positive response to bradykinin, carbachol, potassium chloride, endothelin I	Messier *et al.*, 1994
Cell communication	Functional gap junctions (tested by microinjection of carboxyfluorescein)	Filip *et al.*, 1986
Matrix secretion	Prolyl-4-hydroxylase / Collagen type I staining	Messier *et al.*, 1994; Taylor *et al.*, 2000
	Fibronectin staining	Messier *et al.*, 1994; Flanagan *et al.*, 2003
	Chondroitin sulphate staining	Messier *et al.*, 1994; Flanagan *et al.*, 2003
	Laminin staining	Flanagan *et al.*, 2003
Innervation	Close apposition to motor nerve terminals	Filip *et al.*, 1986
	Proximity to nerves displaying:	
	Neuropeptide Y activity	Marron *et al.*, 1996; Ahmed *et al.*, 1997
	Tyrosine hydroxylase activity	Marron *et al.*, 1996; Ahmed *et al.*, 1997
	Acetylcholineesterase activity	Marron *et al.*, 1996; Ahmed *et al.*, 1997
	Vasoactive intestinal polypeptide (VIP) activity	Marron *et al.*, 1996
	Substance-P activity	Marron *et al.*, 1996; Ahmed *et al.*, 1997
	Calcitonin gene-related peptide (CGRP) activity	Ahmed *et al.*, 1997
Mitogenic factors	Platelet-derived growth factor (PDGF) – mitogenic effect	Johnson *et al.*, 1987
	Basic fibroblast growth factor (bFGF) – mitogenic effect	Messier *et al.*, 1994
	Serotonin (5-HT) – mitogenic effect	Hafizi *et al.*, 2000
Fibroblast characteristics	Incomplete basal lamina	
	Long, thin cytoplasmic processes	
	Intimate association with matrix	
	Well-developed rough endoplasmic reticulum and Golgi apparatus	Filip *et al.*, 1986
	Rich in microfilaments	
	Adherens junction formation	

Table 2 Characteristics of valvar endocardial cells (VECs)

Anatomical or Physiological Feature:	**Evidenced by:**	**Reference:**
Morphology	Cobblestone shape (2-D culture)	Manduteanu *et al.*, 1988
	Regional differences *in situ* (cobblestone/ elongated)	Hurle *et al.*, 1985
	Surface microappendages	Hill and Folan-Curran, 1993
	Marginal folds	Harasaki *et al.*, 1975
Cytoskeleton	Actin staining	Garcia-Martinez and Hurle, 1986
	Vimentin staining	Garcia-Martinez and Hurle, 1986
Metabolic function	Von Willebrand factor (vWf) production	Manduteanu *et al.*, 1988
	Angiotensin converting enzyme (ACE) activity	Manduteanu *et al.*, 1988
	Prostacyclin biosynthesis	Manduteanu *et al.*, 1988
	Endothelial nitric oxide synthase (eNOS) production	Smith *et* al., 1993; Siney and Lewis, 1993
Cell communication	Tight junction formation	Lupu and Simionescu, 1985
	Functional gap junctions (tested by microinjection of carboxyfluorescein)	Lupu and Simionescu, 1985
Ultrastructural features	Lack of Weibel-Palade bodies	Lester *et al.*, 1993
	Plasmalemmal vesicles / caveolae	Filip, 1984; Rajamannan *et al.*, 2002
	Prominent rough endoplasmic reticulum and Golgi apparatus	Manduteanu *et al.*, 1988

Table 3 Principal ECM components of the heart valve layers

Valve layer (Atrialis)	**Main ECM component** (Elastin)	**Function** (Recoil of AV valves during closure)
Fibrosa	Collagen	Tensile strength
Spongiosa	Proteoglycans	Formation of hydrated lattice Resistance against compressive forces
Ventricularis	Elastin	Retention of corrugated collagen structure in aortic valve Recoil of arterial valves during closure

fibre bundles appear to be surrounded by an elastin matrix, which provides interconnections between the collagen fibres (Scott and Vesely, 1995). The glycosaminoglycan (GAG) side-chains of proteoglycan molecules tend to form a gel-like ground substance in which other matrix molecules interact to form permanent cross-links and on which other components are deposited. Human heart valve GAGs consist mainly of hyaluronic acid (HA), followed by dermatan sulphate (DS), chondroitin-4-sulphate (C-4S) and chondroitin-6-sulphate (C-6S), with minimal heparan sulphate (HS), in decreasing concentration (Murata, 1981).

The deposition and subsequent remodelling of ECM components help to determine many of the mechanical characteristics of the heart valve; however, the appropriate biological, chemical and physical cues are not yet completely understood. Both tissue remodelling and renewal observed in the connective tissue of structures such as heart valves are mediated primarily by the matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs). These molecules also participate in a broader spectrum of physiological and pathological processes (Nelson *et al.*, 2000; McCawley and Matrisian, 2001; Galis and Khatri, 2002). Several MMPs, including interstitial collagenases (MMP-1, MMP-13) and gelatinases (MMP-2, MMP-9) and their TIMPs (TIMP-1, TIMP-2, TIMP-3) have been localised in all four heart valves (Dreger *et al.*, 2002). Over-expression of MMPs has also been demonstrated in pathological heart valve conditions (Rabkin *et al.*, 2001). Further stud-

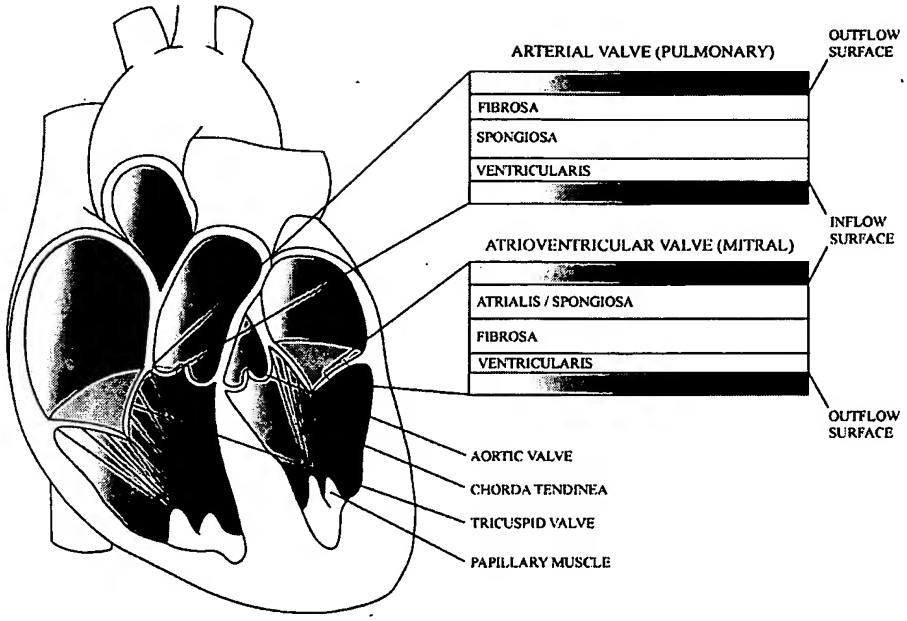


Figure 1 Schematic arrangement of the connective tissue layers through the mid-zone of the heart valve leaflets

ies are necessary to elucidate their mechanism of action and functional implications in valve matrix turnover and tissue morphogenesis.

Each of the three major structural valve components (collagen, elastin and proteoglycans) constitutes a significant proportion of each of the three morphologically distinct and functionally significant layers of the valve leaflet matrix (Table 3). These layers are termed the fibrosa, spongiosa, and ventricularis (Gross and Kugel, 1931), although the arrangement of these layers differs in the AV and arterial valves (Figure 1). The AV valves also possess an atrialis layer, but the atrialis and spongiosa layers will generally be considered together as the spongiosa layer for the purpose of this review.

Fibrosa. The fibrosa layer forms the load-bearing fibrous backbone of the valve leaflet, consisting of sheets of collagen bundles (Kunzelman *et al.*, 1993). These bundles preferentially run in the transverse direction of the aortic valve leaflets and are arranged in a corrugated manner to allow expansion during valve closure (Missirlis and Armeniades, 1977). The fibrosa of the AV valves is continuous with the collagen core of the chordae tendineae (Figure 1) – a network of cord-like support structures anchored to the cardiac papillary muscles (Fenoglio *et al.*, 1972). The chordae make up an important component of the AV valve complex, as they prevent the leaflets from ballooning into the atria during ventricular contraction. The fibrosa layer of the heart valves generally lies towards the outflow surface, and helps maintains proper function in the arterial valves as they are unsupported by chordae tendineae (Christov *et al.*, 1999).

Spongiosa. The spongiosa layer consists of loosely organised connective tissue in a semi-fluid ground substance (Walmsley, 1978). The dominant matrix components of this layer are proteoglycans, with randomly oriented collagen interspersed with multiple fine layers of elastic tissue. GAG side-chains of proteoglycan molecules are highly negatively charged which results in a significant water-binding capacity and thus the formation of a porous gel matrix. Important mechanical functions of the proteoglycans include this matrix hydration, and the ability to resist compressive forces (Culav *et al.*, 1999). The spongiosa layer of the heart valve may absorb stresses generated during valve closure (Kunzelman *et al.*, 1993) and may sustain flexibility despite continuous valve motion (Murata, 1981). This layer may also enable shearing between the fibrosa and ventricularis layers of the aortic valve during loading and unloading.

Ventricularis. The ventricularis layer is much thinner than the other two layers, and is abundant in elastic fibres (Kunzelman *et al.*, 1993). Elastic fibres allow tissues to withstand repeated deformation and reformation (Culav *et al.*, 1999). Elastin is believed to be responsible for maintaining the aortic valve collagen fibre architecture in its neutral corrugated state (Scott and Vesely, 1995). It is also evident that the side of the valve which faces *against* the direction of blood flow (ventricularis in arterial valves, spongiosa in AV valves) contains a higher proportion of elastin than the opposite layer, and its flexibility allows the valve leaflets to recoil during valve closure (Christov *et al.*, 1999).

In order to meet the extraordinary demands placed upon it, the valve possesses incredible strength, durability and flexibility due to its composite laminated structure. It will be crucial to recreate this layered structure in a LAHVA to maintain proper functionality amidst the turbulent conditions of the cardiac cycle. Any analysis of normal valve function must take into account the cellular organisation of the valve as well as the reciprocal interactions between the cells and their surrounding matrix. Knowledge gained from such

studies is central to the design and development of a living replacement valve employing a tissue-engineered approach.

Tissue Engineering Approach to Heart Valve Replacement

Tissue engineering approaches emerged during the last 15 years in response to limitations associated with tissue and organ transplantation, with the scarcity of available donor tissue being perhaps the most significant limiting factor (Fuchs *et al.*, 2001). The primary goal of tissue engineering is the 'restoration of function through the delivery of living elements which become integrated into the patient' (Vacanti and Langer, 1999). Tissue engineering approaches to the construction of a heart valve, or any tissue or organ, typically rely on three essential components (Figure 2): *cells*, which will ultimately form the new tissue; *scaffolds*, designed to maintain the cells in a three-dimensional environment at the implantation site, and *signals* that guide the gene expression and ECM production of the cells during tissue development.

Cells

Establishing a reliable cell source is a key to effective tissue regeneration (Langer and Vacanti, 1999). A variety of cell sources is applicable to tissue-engineered structures, each with associated advantages and disadvantages (Heath, 2000). One possibility is autogeneic cells, harvested from patients themselves, which would not elicit an immune reaction following re-implantation. However, in diseased states or with geriatric patients, autogeneic cells may not be an appropriate transplantation source (Heath, 2000). They would also be unavailable 'off-the-shelf' for immediate use clinically (Nerem and Seliktar, 2001).

Recent developments in stem cell research have had a significant impact on the progress of tissue engineering (Chapekar, 2000). This research has focussed particularly on multipotent (capable of forming a number of cell types) adult stem cells and totipotent (capable of forming all cell types) embryonic stem cells. However, a clearer understanding of the factors involved in their differentiation, as well as preservation of gene expression and phenotypic function *in vivo* is critical to maintain normal structure and function in a LAHVA.

The remaining possibilities for a LAHVA cell source include xenogeneic or allogeneic cells, although xenogeneic cells carry a risk of transferring animal viruses to humans (Nerem and Seliktar, 2001). There may be a possibility, however, to alter allogeneic cells genetically to reduce antigenicity or prevent host rejection (Heath, 2000). Regardless of cell source, the cells composing a tissue-engineered structure must express the appropriate genes and maintain the appropriate phenotype in order to preserve the specific function of the tissue (Kim and Mooney, 1998).

Scaffolds

Exogenous ECM scaffolds are designed to immobilise the appropriate cell populations at the implantation site of a tissue-engineered structure, and also to provide mechanical support until newly-formed tissue has been laid down,

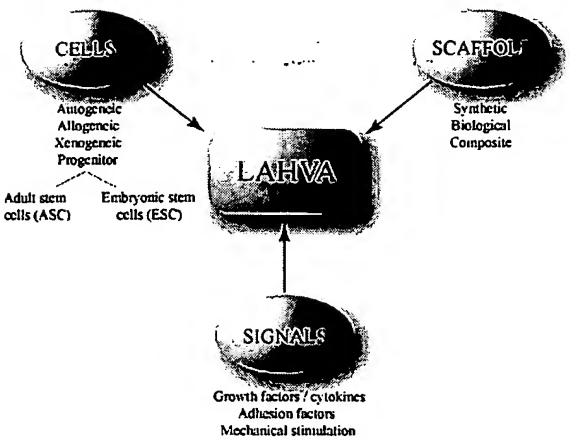


Figure 2 The principal building blocks for LAHVA fabrication

organised and become structurally stable (Putnam and Mooney, 1996). The traditional concept of tissue engineering involves the use of three-dimensional polymeric scaffolds as cell transplant devices. Once the cells are seeded on the scaffolds *in vitro*, the resulting three-dimensional construct can be implanted *in vivo* at the site of injury or disease. Implanted cells can then use the body's natural structural cues to promote organisation, growth and development of the replacement 'tissue'. The polymeric scaffolds are designed to degrade or hydrolyse *in vivo* as the implanted cells produce and organise their own extracellular matrix network, such that eventually only newly synthesised, functionally integrated tissue remains at the transplantation site. The two principal choices of scaffold currently under investigation are synthetic polymeric scaffolds or biological polymeric scaffolds.

Signals

Successful tissue regeneration may not always be achieved 'simply' by combining cells and scaffolds. The gene expression of cells and tissue formation can be regulated or promoted by the addition of growth factors, cytokines or hormones. A drug delivery feedback system can exploit this method of biochemical signalling, by controlling growth factor release at the site of tissue regeneration (Pandit *et al.*, 2000; Tabata, 2000).

Cell adhesion molecules also impart a level of signalling that controls the regulation of gene expression and cellular organisation. Bioactivity of a synthetic polymer scaffold can be modified with covalently-attached adhesion peptide sequences, such as arginine-glycine-aspartic acid (RGD) or tyrosine-isoleucine-glycine-serine-arginine (YIGSR) (Hubbell, 1995). Such sequences are present on a number of ECM molecules, forming a physical link between the ECM and the cells via integrin cell surface receptors (Van der Flier and Sonnenberg, 2001). Incorporated sequences function to improve cell adhesion, spreading and higher functions, whilst also enabling integrin triggering of a number of cell signalling pathways critical to tissue development (Kim and Mooney, 1998).

In vitro methods of mechanical stimulation or physical signalling have also been shown to improve cell and tissue growth. This has led to the design and development of bioreactor systems for the *in vitro* pre-conditioning of tissue-engineered structures (Barron *et al.*, 2003); tissue formation thus takes place in an environment closely mimicking that of the transplant site.

Current progress towards a LAHVA

The design of LAHVAs incorporating cells, scaffolds and signals presents a unique challenge to researchers in the field. The optimum design will possess the following characteristics: an anatomical structure comparable to native valve; physiological function; biomechanical adaptability; reparative ability and growth potential. The valves must be able to open and close synchronously at a frequency of approximately 1Hz, resulting in bending deformations, and be exposed to the turbulent hemodynamics experienced within the heart (Mann and West, 2001).

Significant progress has been made to date in the tissue engineering of heart valve structures, with researchers concentrating their efforts on the ideal source of cell populations, the use of cell-seeded scaffolds and the promotion of tissue growth using various signals, in particular *in vitro* mechanical stimulation. The results of selected studies are summarised in Table 4 (to be found in supplemental file 'v006a04s.pdf').

Populations investigated as cell sources for a LAHVA

The concept of a LAHVA was first presented by Shinoka *et al.* (1995), who constructed valve leaflets using both allogeneic and autogeneic cells seeded on a biodegradable polymeric scaffold. A mixed population of smooth muscle cells and fibroblasts representing VICs was isolated from ovine femoral artery and subsequently seeded into scaffolds composed of polyglactin (PGLA) and polyglycolic acid (PGA). The constructs were then seeded with a monolayer of femoral artery endothelial cells representing VECs. The single leaflet structures were transplanted in the low-pressure pulmonary position in ovine models. The autogeneic structures performed better than allogeneic, the latter evoking inflammatory responses and infectious complications in the animal hosts. The study did however demonstrate the feasibility of LAHVA leaflet construction.

Subsequent studies also utilised ovine femoral artery-derived cells to fabricate valve leaflet constructs (Breuer *et al.*, 1996; Shinoka *et al.*, 1996). However, the use of the femoral artery is not ideal as an autogeneic cell source, due to the added complications of limb ischaemia from disruption of the lower limb arterial blood supply. To address this problem, Shinoka *et al.* (1997) designed a study comparing dermal fibroblasts to arterial myofibroblasts* as cells of origin for a LAHVA. Leaflets derived from dermal fibroblast were much thicker, more contracted and less organised than those derived from arterial myofibroblasts. The authors suggest that cells of mesodermal origin (such as arterial myofibroblasts) may provide more specialised phenotypic properties than ectodermally-derived skin fibroblasts, making mesodermal cell populations more suitable for the construction of a LAHVA.

Schnell and co-workers (2001) evaluated myofibroblasts derived from human saphenous vein as an alternative cell source for the tissue engineering of cardiovascular structures. The choice of peripheral veins represented a more clinically realistic cell source than the arterial structures previously used, and can be harvested with minimally invasive surgery. The myofibroblasts were cultured on polyurethane scaffolds for 6 weeks, following which they were shown to be viable and confluent. Collagen production was increased in saphenous 'neo-tissue' compared to that constructed using human aortic myofibroblasts, while mechanical testing also demonstrated superior stability in the saphenous structures. However, as with most previous studies using arterial cells, the production of other important structural and regulatory molecules remains to be elucidated.

Carotid artery-derived myofibroblasts have also been harvested for the construction of a LAHVA (Stock *et al.*, 2000; Sodian *et al.*, 2000a; Hoerstrup 2000a); however, as with all other previous cell types isolated for the same purpose, this requires the sacrifice of an intact tissue structure and requires invasive surgery. In the search for an alternative cell source, one group demonstrated the possibility of using autogeneic umbilical cord cells (Kadner *et al.*, 2002a). The isolated cells represent a mixed population from umbilical cord artery, vein and the surrounding Wharton's jelly. Absence of endothelial cells was confirmed *in vitro*; cells demonstrated features of myofibroblast-like differentiation, such as expression of alpha smooth muscle actin, vimentin, and deposition of collagen types I and III. The cells were shown to attach well to biodegradable scaffolds, and formed a layered tissue-like structure comparable to scaffolds seeded with vascular cells (Hoerstrup *et al.*, 2000a). Deposition of collagen types I and III was demonstrated, although there were low levels of GAGs produced, and no report of elastin production. There are also a number of other limitations to this study; although Wharton's jelly myofibroblasts, the umbilical cord artery or vein-derived cells on their own may represent a promising cell source, the authors present a study with a 'mixed' population of cells from all three structures. There is also limited immunohistochemical characterisation of this 'mixed' population, and further characterisation studies of pure cell

*Many papers have misleadingly referred to cells isolated from veins and arteries as 'myofibroblasts', with minimal characterisation. It has been proposed that most examples of cells cited in the literature as being myofibroblasts and seen in normal, non-traumatised, non-pathological tissues may be pericytic or smooth-muscle in nature (Eyden *et al.*, 1994). Indeed, the vascular-derived cells are more likely to be smooth muscle cells, which are generally either synthetic or contractile. This again highlights the importance of extensive cell characterisation, and a standardisation of the cell terminology is of paramount necessity. Solely for the purpose of this review, the authors will cite the terminology used by the original authors.

lines from each source will be necessary to significantly evolve this approach.

Mesenchymal stem cells (MSCs) are present in adult bone marrow, and have been demonstrated to develop into a variety of connective tissues, including bone, cartilage, muscle and fat (Pittenger *et al.*, 1999; Caplan and Bruder, 2001). With this in mind, Kadner and colleagues investigated the feasibility of creating functional LAHVAs using human bone marrow stromal cells (Kadner *et al.*, 2002b; Hoerstrup *et al.*, 2002). Cell populations are easily harvested from a patient using simple bone marrow puncture, avoiding the sacrifice of an intact blood vessel. Isolated cells were partially characterised using a number of myofibroblast markers, and, like umbilical cord cells, were shown to express alpha smooth muscle actin and vimentin, and produce collagen types I and III. Biodegradable polymeric scaffolds cultured with MSCs *in vitro* demonstrated an organised internal structure and mature tissue development. Despite the encouraging results, it is essential that the bone marrow stromal cells differentiate into the appropriate cell type in the scaffold, and that they remain differentiated *in vivo*, in order to ensure long-term function and durability of the implanted LAHVA (Perry *et al.*, 2003). Further investigation into the mechanisms and signals that direct bone marrow stromal cell differentiation are warranted before introduction in the clinical setting.

Although some promising results have been obtained using the aforementioned cell types, the ideal cell replacements for a LAHVA would be VICs and VECs themselves. A recent report discusses the use of heart valve leaflet biopsy samples from an ovine model as a tissue source for the cultivation of VICs for use in LAHVA fabrication (Maish *et al.*, 2003). In most cases, the biopsy procedure did not appear to compromise leaflet function, with the majority of animals showing intact valves with normal leaflet anatomy post-mortem (9 out of 13). Long-term follow-up studies are required (the maximum investigation period was 17 weeks); however, it is unlikely that enough cells could be isolated and cultured from a small biopsy to be of clinical use. It is also known that cell numbers decrease with age, making this technique even less feasible in the older patient. The risks involved in valve biopsy may also be too high to justify human trials.

The search for an ideal replacement for both cell types is ongoing, with other potential sources of interest including circulating endothelial and smooth muscle progenitor cells (Rafii, 2000; Simper *et al.*, 2002). However, all current techniques assume that transplanted cell populations achieve the same distribution and differentiation pattern of cells in the native valve (Bertipaglia *et al.*, 2003). In previous studies, there is limited data regarding the phenotypic profile of these cells once they are implanted into the scaffolds, and future studies will need to provide extensive characterisation profiles of tissues both prior to implantation and during long-term follow-up studies. It is highly probable that both embryonic and adult stem cells will become valuable resources for construction of a number of tissues *ex vivo*, including heart valves.

Scaffolds investigated as ECM templates for a LAHVA

Synthetic biodegradable polymeric scaffolds. The work by Mayer *et al.* at the Children's Hospital, Boston in the mid-to-late 1990s involved the application of the biodegradable polymers PGA and polylactic acid (PLA), and composites of these polymers (PGLA), in the development of a LAHVA (Shinoka *et al.*, 1995; Breuer *et al.*, 1996; Shinoka *et al.*, 1996; Zund *et al.*, 1997). The scaffolds were composed of a PGLA woven mesh sandwiched between two non-woven PGA mesh sheets. The constructs were first seeded with arterial myofibroblasts, secondly with arterial endothelial cells, and were subsequently transplanted as a single leaflet into the pulmonary position in an ovine model. Seeded cells were shown to persist in the structure after 6 weeks *in vivo* (Shinoka *et al.*, 1996). Histological examination of the leaflet structures post-mortem revealed a cellular architecture and tissue structure similar to that of native valve, although inflammatory responses and complications due to infection in the animal study were evident (Shinoka *et al.*, 1995). The scaffold also persisted for at least 6 weeks after implantation. There was evidence for elastin and collagen production in the leaflets, which had mechanical properties similar to native leaflets (Shinoka *et al.*, 1996). The success of these studies was limited, however, as the PGA-PLA polymers proved to be too immalleable to function as flexible valve leaflet structures. Kim and associates (2001) attempted to conduct a study to compare the biological responses of autogeneic cell-seeded PGA-PLA scaffolds and an acellular PGA-PLA scaffold by implanting both structures in the pulmonary position of the same animal. However, they failed to achieve long-term survival in animals with double valve leaflet replacement. They attributed this to heart failure caused by high-grade valvar insufficiency.

The high initial stiffness and thickness using PGA-PLA scaffolds warranted the investigation of new polymers. Stock and colleagues (2000) evaluated the use of polyhydroxyoctanoate (PHO) as part of a composite scaffold for the creation of a LAHVA. The fabricated valved-conduit consisted of four different biomaterials: the conduit wall was composed of a layer of non-porous PHO film (240μm thick) sandwiched between two layers of non-woven PGA felt (each 1mm thick). The leaflets consisted of a monolayer of porous PHO (120μm thick), and were sutured to the wall of the conduit using polydioxanone sutures. Microscopical examination after explantation revealed a uniformly organised, fibrous tissue with significant amounts of stainable collagen and proteoglycans, but without stainable elastin. However, the PHO scaffold was still evident in the conduit and leaflets after 24 weeks, suggesting a much longer degradation profile than PGA or PLA, and this extended period could have the potential to augment host-tissue reactions. The study was also limited to implantations in the low-pressure pulmonary position.

An additional study involved moulding a porous PHO scaffold into a trileaflet valved-conduit through a thermal processing technique, with subsequent seeding of ovine carotid artery myofibroblasts and jugular vein endothelial cells (Sodian *et al.*, 2000b). The constructs, devoid of

polydioxanone sutures, were implanted into the pulmonary position in ovine models, and were harvested after 1 - 17 weeks. All valve constructs opened and closed synchronously, and showed an increase in inner diameter and length. This could not conclusively be attributed to actual tissue growth, and may simply have been dilatation of the construct. Scanning electron microscopical studies demonstrated a smooth flow surface on the leaflets, although they did not demonstrate a confluent endothelium using histological techniques. This limitation could affect the long-term durability of the structures. There was evidence for collagen and GAG deposition, but the constructs were devoid of elastin. All valve constructs demonstrated mild stenosis and regurgitation, and there was evidence for inflammatory reaction. The scaffold material remained after 17 weeks *in vivo*, and further long-term studies are required to monitor complete scaffold degradation.

PHO scaffold was evaluated as an alternative to PGA-PLA, but demonstrated prolonged degradation time *in* vivo, and was not sufficiently replaced by neotissue after 24 weeks (Stock *et al.*, 2000). Hoerstrup and colleagues (2000a) developed a novel composite scaffold material consisting of PGA coated with a thin layer of poly-4-hydroxybutyrate (P4HB) – a flexible, thermoplastic polymer with a more rapid degradation time than PHO (Martin and Williams, 2003). Trileaflet heart valve scaffolds were fabricated from the composite material using a heat-application welding technique. Myofibroblasts and endothelial cells from ovine carotid artery were seeded onto the scaffolds, which were then cultured in a bioreactor for 14 days (Hoerstrup *et al.*, 2000a). The conditioned constructs were subsequently implanted into the same lambs from which the cells were harvested, where they remained for up to 20 weeks. After *in vitro* conditioning, constructs showed increased ECM synthesis, more organised internal structure and improved mechanical properties over static controls. The leaflets demonstrated a layered architecture after 16 and 20 weeks, with a loose, spongy layer containing elastin and GAGs on the inflow (ventricular) surface, and a more fibrous layer containing primarily collagen on the outflow (arterial) surface. However, the valves showed moderate regurgitation at 20 weeks, and only partial endothelial cell coverage of the leaflet surface.

Decellularised tissue scaffolds. Decellularisation approaches may reduce the immune response of the host to bioprosthetic valves and generate natural materials for use in tissue engineering applications (Schmidt and Baier, 2000). The acellular biological matrix that remains offers a physiological template, which holds the potential for endothelialisation by surrounding host VECs and repopulation by local VICs. Theoretically, VECs will form a non-thrombogenic surface layer, while VICs will induce matrix turnover in the valve. This process would ultimately transform the allogeneic or xenogeneic acellular matrix into a primarily autogeneic, living valve tissue, with concurrent reduction in immune response. There are various methodologies available for decellularisation of heart valve tissue, including detergent treatments, enzymatic digestion, hypo-/hypertonic immersion and sonication.

Wilson and colleagues (1995) developed a cell extraction process for decellularising heart valves using both detergent (Triton X-100) and enzymes (DNAse, RNAse). The process removes cell membranes, nucleic acids, lipids, cytoplasmic structures, and soluble matrix molecules, while retaining the collagen and elastin ECM suitable for recellularisation (Zeltinger *et al.*, 2001). After one month of follow-up using decellularised allografts in a canine model (Wilson *et al.*, 1995), explant histology demonstrated no inflammatory process, partial VIC infiltration at the valvar base, and partial endothelialisation. Long-term calcification studies were not performed.

Bader and colleagues (1998), employing the decellularisation strategy of Wilson *et al.* (1995), demonstrated reseeding of acellularised porcine aortic valves with human endothelial cells, resulting in a confluent surface monolayer of viable cells. Endothelialisation of LAHVAs is an important preconditioning factor, in order to reduce thrombogenic risk. These authors demonstrated a largely acellular structure across the thickness of the leaflet. The collagen network appeared wavelike, as in normal valve tissue; however, there were large interfibrillar spaces that may have affected the mechanical properties of the tissue. It was also impossible to exclude the presence of cellular remnants in the tissue structure. This may be an important factor, as cellular remnants have been associated with calcification in microscopic investigations (Valente *et al.* 1985, Schoen *et al.* 1985). The extraction of cellular remnants should also minimise the immune response (Schmidt and Baier, 2000).

Bader and colleagues extended their studies to the *in vivo* application of an acellular matrix valve scaffold conditioned by tissue engineering *in vitro* using autogeneic cells (Steinhoff *et al.*, 2000). Carotid artery myofibroblasts and endothelial cells were added sequentially to acellularised pulmonary valve conduits and were implanted into ovine models. The valve leaflets were completely endothelialised at 4 weeks, with the endothelium remaining confluent at 12 weeks after implantation, and had been infiltrated by a dense population of myofibroblasts. However, there was evidence for subvalvular calcification and inflammatory reaction. There was also an increased thickening of the valve leaflets; long-term studies were not undertaken.

The decellularisation approach is the basis for the commercially available SynerGraft™ valve manufactured by CryoLife Inc., USA (O'Brien *et al.*, 1999). The SynerGraft™ decellularisation process involves cell lysis in sterile water, enzymatic digestion of nucleic acids (RNase and DNase enzymes in an unspecified buffer), followed by a multi-day isotonic washout period. The process was developed to replace the use of glutaraldehyde cross-linking to limit xenograft antigenicity. Histological examination after 150 day implantation in porcine models revealed intact leaflets with ingrowth of host myofibroblasts and no evidence for calcification. However, early failure of the valve has been reported in human trials (Simon *et al.*, 2003). The SynerGraft™ matrix elicited a strong inflammatory response which was non-specific early on and was followed by a lymphocyte response. Structural failure or rapid degeneration of the valves occurred within one year. There was no host cell repopulation of the valve matrix, and evidence for calcific deposits and cellular remnants pre-implantation may indicate manufacturing problems.

The ideal heart valve decellularising agent remains unknown. In a recent effort to determine the most efficient decellularising agent from a panel of candidates (Triton X-100, sodium dodecyl sulphate (SDS), sodium deoxycholate, MEGA 10, TnBP, CHAPS and Tween 20), Booth and colleagues (2002) demonstrated that only SDS (0.03 - 1%) or sodium deoxycholate (0.5 - 2%) resulted in total decellularisation after 24 hours. However, the possible toxicity of decellularisation reagents remains a factor for investigation; more detailed characterisation studies of the matrix scaffold are crucial to determine appropriate matrix composition and complete elimination of cellular remnants. Biomechanical properties of decellularised valve tissue matrices also need to be analysed.

Natural biodegradable polymeric scaffolds. A number of naturally occurring biodegradable polymers have been investigated as potential LAHVA matrix structures, as they offer a more native environment than synthetic structures to the cells. Acellular small intestinal submucosal (SIS) matrix has been isolated to construct tissue substitutes (Badylak *et al.*, 1989; Badylak *et al.*, 1998). Matheny and colleagues (2000) have applied porcine SIS matrix as a resorbable scaffold for pulmonary valve leaflet replacement in porcine models. Analysis of explanted constructs revealed resorption of the submucosal matrix, progressive replacement with fibrous connective tissue and vascularisation similar to host leaflets. This approach appeared to yield complete resorption of the implanted scaffold in comparison to decellularised valve matrix applications.

The use of fibrin gel as a natural, autogeneic scaffold in heart valve tissue engineering has been demonstrated by Ye and associates (2000a), and has been the subject of investigation for the development of cardiovascular 'tissue equivalents' by Tranquillo and colleagues (Grassl *et al.*, 2002; Neidert *et al.*, 2002). Fibrin gel is a biodegradable polymer which can be produced from the patient's own blood. In the study by Ye and colleagues (2000a), cell-fibrin gel structures were constructed using human aortic myofibroblasts suspended in a solution of fibrinogen, thrombin and calcium chloride ($CaCl_2$) which was allowed to polymerise at 37°C. The growth media was supplemented with L-ascorbic acid 2-phosphate – a potent promoter of collagen synthesis (Grinnell *et al.*, 1989), and varying concentrations of aprotinin, a protease inhibitor, to adjust and control the rate of degradation. Microscopical studies demonstrated homogenous cell distribution and collagen synthesis; degradation was controlled by altering the aprotinin concentration. A moulding technique has also been developed for the application of this compound to trileaflet heart valve fabrication (Jockenhoevel *et al.*, 2001a). An adjustable mould consisting of 'aortic' and 'ventricular' stamps was constructed from aluminium, and used to cast a trileaflet fibrin gel structure seeded with myofibroblasts within 1 hour. The tissue could withstand suturing, although its mechanical integrity was insufficient for direct implantation. Shrinkage of the gel structure also presents a complication. However, the fibrin gel model has a number of advantages: the scaffold itself can be produced directly from a sample of patient's blood eliminating the risk of rejection; the rate of degradation can be controlled and a homogenous cell distribution can be achieved upon polymerisation.

As fibrous long-spacing collagen provides most of mechanical and tensile strength of the heart valve, a valve construct fabricated using a natural collagen scaffold is quite a logical choice. Collagens are weakly immunogenic compared to other structural proteins, due to a large degree of homology across species, and are biodegradable on account of their proteinaceous nature (Chevallay and Herbage, 2000). Collagen scaffolds have been investigated for the production of heart valve-like tissue using human and porcine-derived cells (Rothenburger *et al.*, 2001; Flanagan *et al.*, 2002; Taylor *et al.*, 2002). Rothenburger and colleagues used a cryogenically-engineered collagen type I matrix derived from bovine skin tissue, with a homogenous pore size of 40μm and a porosity of 98%. The matrix was cut into disc structures and sequentially seeded with either human or porcine aortic smooth muscle cells. The structures were then seeded with porcine aortic endothelial cells and maintained in culture for 28 days. A tissue-like morphology was observed in all samples, characterised by several layers of cells with newly synthesised ECM components. Proteoglycans and the ECM proteins fibronectin and thrombospondin were also localised.

Ye and colleagues (2000b) took another approach to the construction of tissue using 'natural' materials by producing completely human autogeneic tissue without the use of a supporting scaffold. Myofibroblasts were cultured in large culture dishes in medium supplemented with L-ascorbic acid 2-phospate to promote extracellular matrix production. The cell sheets subsequently formed were folded into quadrilaminar sheets and mounted on customised culture frames. They were then cultured for a further 4 weeks and demonstrated a multilayer tissue pattern, with active viable cells surrounded by extracellular matrix.

Adequate, uniform and reproducible cell seeding of both natural and synthetic polymeric scaffolds remains a difficulty in the field. In an effort to improve scaffold cell-seeding methods, Ye and colleagues (2000c) have demonstrated the possibility of coating scaffolds with an autologous immuno-compatible ECM material; Zund and colleagues (1999) aimed to improve cell-seeding methods by optimising the cell seeding interval (i.e. the length of time between subsequent seeding procedures). Their results suggest that longer seeding intervals of 24- to 36-hours result in higher cell attachment and cell growth on PGA scaffolds, this may have been due to 'pre-conditioning' of the seeded scaffolds by cells previously laid down. Optimisation of rapid seeding techniques will be important in LAHVA fabrication, as it maximises the use of donor cells, hastens the proliferation and subsequent differentiation of cells, decreases the time in culture, and provides a uniform distribution of cells (Vunjak-Novakovic *et al.*, 1998). Advances in the modification of scaffold surfaces to enhance cell adhesion and subsequent function will be necessary for short-term *ex vivo* culture of tissues prior to implantation.

It is not clear whether natural or synthetic scaffolds will ultimately prove to be more appropriate for LAHVA development, as there are still certain limitations associated with both. The optimum heart valve scaffold should display an

accurate, anatomical spatial reconstruction of the valve to be replaced. The design of a replacement aortic valved-conduit, for example, will require the inclusion of three dilated pouches alongside the 'cusp-like' leaflets to approximate the sinuses of Valsalva; AV valve designs may require the addition of other components of the AV valve apparatus, including chordae tendineae and papillary muscles (Bursac *et al.*, 1999; Zimmermann *et al.*, 2002). Using stereolithography, Sodian and colleagues (2002) fabricated plastic models with an exact spatial representation of human aortic and pulmonary valve. These models were then used to fabricate heart valve cell scaffolds using poly-3-hydroxyoctanoate-co-3-hydroxyhexanoate (PHOH) and P4HB, which were shown to function well in a pulsatile flow bioreactor under both normal and supranormal flow and pressure conditions. The choice and design of scaffolds will be integral to the successful clinical outcome of a LAHVA. As well as conforming to a suitable anatomical shape, heart valve scaffolds will be required to possess both tensile and elastic properties. The scaffolds should be biocompatible, bioabsorbable or remodelable, and should provide a suitable template for facilitating development of new tissue. Scaffold permeability is also vital for the control of cell nutrition and removal of waste products.

Signalling factors investigated in LAHVA development

There is a dearth of research into the application of growth factors, mitogenic factors or adhesion factors in scaffolds and matrices used to develop a LAHVA. However, significant progress has been made in the development of *in vitro* methods of mechanical stimulation or physical signalling to improve cell and tissue growth prior to implantation (Hoerstrup *et al.*, 1999; Weston *et al.*, 1999; Hoerstrup *et al.*, 2000b; Sodian *et al.*, 2001; Weston and Yoganathan, 2001; Zeltinger *et al.*, 2001; Jockenhoevel *et al.*, 2002; Dumont *et al.*, 2002; Perry *et al.*, 2003; Engelmayr *et al.*, 2003).

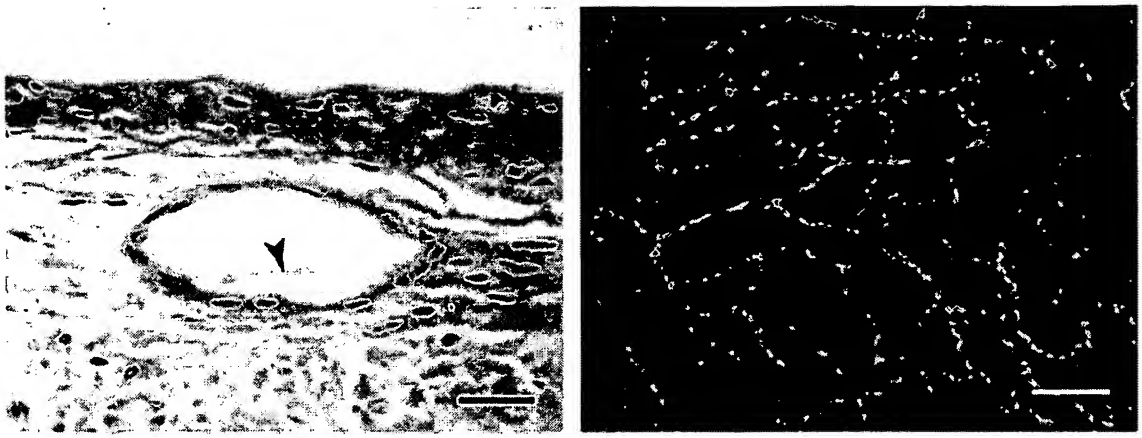
One method of mechanically stimulating cells to induce tissue morphogenesis is maintaining them in culture under isometric strain (Stopak and Harris, 1982). A number of groups report increased collagen synthesis and tissue organisation in LAHVA scaffolds mounted in cultures frames that provide isometric strain (Hoerstrup *et al.*, 1999), border-fixed fibrin gel structures (Jockenhoevel *et al.*, 2001b) and cyclically-strained scaffolds (Mol *et al.*, 2003).

The mechanical properties obtained by Niklason *et al.* with tissue-engineered vascular constructs cultured under pulsatile flow conditions (Niklason *et al.*, 1999) prompted a number of other investigators to develop bioreactor systems to improve the mechanical structure of LAHVAs. Jockenhoevel and colleagues designed and constructed a flow system to investigate the effects of laminar flow conditions on ECM formation and tissue development in cell-seeded synthetic biodegradable polymer scaffolds (Jockenhoevel *et al.*, 2002). The system was efficient at maintaining sterile culture conditions, and extracellular matrix production was significantly higher than static controls. Current experiments in the design and development of a system specific for dynamic culture of fibrin and collagen gel LAHVA structures are ongoing (Flanagan and Jockenhoevel, unpublished results).

Pulsatile flow systems have also been engineered to condition both myocardial patch tissue and trileaflet heart valve tissue *in vitro* (Hoerstrup *et al.*, 2000b; Sodian *et al.*, 2001). The pulse duplicator system of Hoerstrup and colleagues was designed to grow LAHVAs under gradually increasing flow and pressure conditions in order to mirror the physical stimulation encountered *in vivo* (Hoerstrup *et al.*, 2000b). The conditioned structures demonstrated a much higher deposition of ECM, improved tissue organisation and better mechanical properties than those grown in static conditions. Zeltinger *et al.* (2001) demonstrated efficient colonisation of decellularised porcine valve scaffolds with human dermal fibroblasts using a pneumatic flow bioreactor apparatus. The application of pulsatile fluid flow was shown to stimulate ECM production and cell proliferation, improving the structural integrity of the LAHVA.

A major drawback with all of these pulsatile flow systems, however, is the limit to the number of test samples (i.e. only one LAHVA sample can be conditioned at a time), which does not offer statistically significant results. Results are also limited to a combination of mechanical stimuli, rather than observing the effects of single stimuli, such as laminar shear and isometric tension studies previously undertaken (Hoerstrup *et al.*, 1999; Jockenhoevel *et al.*, 2002). A recent bioreactor design allowed studies of the effects of cyclic three-point flexure on LAHVA structures, and incorporated a large test sample size to demonstrate significant results (Engelmayr *et al.*, 2003). The investigators report fatigue and fracture in acellular biodegradable polymer scaffolds cultured under dynamic flexure conditions; this may prove to have important consequences in the design and selection of potential LAHVA scaffold candidates.

Although significant progress has been made, all LAHVAs constructed to date lack the mechanical strength required for functional performance in the anatomical position. All previous studies have been directed towards the design of LAHVAs for transplantation at the low-pressure pulmonary valve site. A clearer understanding of the mechanical environment of all four heart valves, in particular those of the systemic circulation, will lead to the development and production of improved biomimetic environments for the *in vitro* pre-conditioning of LAHVAs. Cell response mechanisms to mechanical stimuli are unclear (MacKenna *et al.*, 2000) and a better understanding will permit the design of more efficient biomimetic protocols. Cell death caused by dynamic bioreactor culture conditions will also need to be investigated, and conditions optimised to minimise cell death. Gene therapy studies have the potential to be exploited in order to promote the expression of suitable mitogenic, angiogenic or neurogenic factors in a LAHVA (Yla-Herttuala and Martin, 2000; Musgrave *et al.*, 2002) to enhance development of a more 'native' heart valve tissue in concert with mechanical stimulation studies.



3a 3b

Figure 3 Both vascular elements **(a)** and neural elements **(b)** have been demonstrated in the heart valve interstitial matrix: **(a)** blood vessel embedded in the atrialis/spongiosa layer of the porcine mitral valve containing red blood cells (arrowhead) (haematoxylin & eosin staining). Scale bar 50μm; **(b)** dense adrenergic nerve plexus situated in the anterior leaflet of the rat mitral valve (sucrose-potassium phosphate-glyoxylic acid staining). Scale bar 30μm.

Conclusion

Heart valve tissue engineering holds great promise for improved treatment of valve disease. Significant advances have been made since its inception in the early 1990s, however, the field remains in its infancy and many issues remain to be addressed and resolved. Primary amongst these is our limited understanding of the normal heart valve, in particular normal valvar cell biology (including gene expression and regulation) and normal valvar ECM composition. A blueprint for LAHVA fabrication can only be achieved through substantial advances in our understanding of basic heart valve biology. Identification of the appropriate transplantation cell types and scaffolds remain the key strategies. Data concerning VECs remains limited, thus characterisation of these cells may take precedence over that of VICs. Furthermore, the presence of vascular (Figure 3a) and in particular neural elements (Figure 3b) in the AV valves requires further research to determine their role in normal valve function. It may be possible to control vascularisation and neurogenesis in a LAHVA by investigating, characterising and incorporating angiogenic and neurogenic factors. A thorough understanding of embryonic and fetal heart valve development may permit the control of heart valve tissue morphogenesis both *in vitro* and *in vivo*. As developmental studies improve, the field should acquire an improved understanding of the mechanisms that may allow for reproducible construction of clinically acceptable heart valves *ex vivo*. It would also allow accurate predictions about valvar morphogenic events under specified conditions, allowing advances to be made in the design and development of suitable bioreactor systems. Other key technological advances are required to enable the transition from experimental model to clinical reality, and will require the cooperation of researchers from a broad spectrum of disciplines. Consistent manufacture of LAHVAs and adequate assessment standards will pose equally challenging factors in this exciting area of research.

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Discussion with Reviewers

P. Taylor: The pressures during valve development are not the same as the maintenance pressures during life. What are the authors views on the flow/pressure conditions required to tissue engineer an aortic valve in a bioreactor? Should increasing pressures be used?

Authors: Fetal blood pressure increases gradually with ongoing maturation, and although there is limited data available, it has been suggested that complete development of all four heart valves takes place under pressure values below 10mmHg, with further remodeling of the structures up to 20 weeks gestation under increasing heart rate (Stock and Vacanti, 2001). A number of previous heart valve bioreactor studies have used pressure ranges increasing above late gestation fetal aortic pressure, with upper values of 55-70mmHg (Hoerstrup *et al.*, 2000a; Hoerstrup *et al.*, 2002). Although some promising results have been obtained, it would be interesting to determine whether or not pressure values closer to those during valve development would improve tissue structure further. Inappropriately high values may inhibit cell proliferation, increase cell-scaffold detachment and may even increase cell death. More gradual increases in pressure over longer time periods, similar to those encountered *in utero*, may also prove beneficial and may establish a more positive feedback mechanism for tissue development. However, different cell sources and scaffold polymers are likely to be influenced in different manners by varying pressure ranges, even if cultured in identical bioreactor systems; therefore, the problems associated with inadequate valve tissue development can only be corrected with all factors taken together.

S. Jockenhövel: A general point of discussion is the expected market for tissue engineered heart valve prostheses. In my opinion the number of potentially needed implants is significantly over-rated. The high production costs will lead to strict indications e.g. for paediatric heart surgery or for the treatment of acute endocarditis.

Authors: The number of necessary implants may not strictly be over-rated, however, the phenomenal costs that will be involved in quality control processes, and in packaging, sterilization and storage of tissue-engineered heart valves, may certainly limit the scale-up of manufacturing processes early on. The preliminary target market for tissue-engineered heart valves is the paediatric population, where these valves would theoretically grow and remodel with the patient and would eliminate the need for successive reoperations. Once a proof of application and efficacy has been established in this market, manufacturing processes can be scaled up to supply <60years market. As the market size increases, production costs will decrease; this may particularly benefit patients in developing countries where rheumatic fever and rheumatic heart disease continue to have a high prevalence rate.

S. Jockenhövel: The current investigations in that field are encouraging – but we have to take care not to implant a tissue engineered heart valve too early. Animal models like sheep are not ideal models concerning endothelialization and hemostasis and might lead to an early failure of the prostheses in human clinical use like we have seen in tissue engineered small calibre vascular grafts.

Authors: Certainly the SynerGraft™ valves have shown that animal models have failed to predict the failures in humans (Simon *et al.*, 2003). Animal experimental models can normally be encouraging but it is very difficult to translate the same success rate to humans. With regard to the poor success rates in animal models of heart valve tissue engineering, we strongly feel that it is the fundamental heart valve biological issues that need to be addressed, and significantly more *in vitro* research must be carried out. Also, the same test models have been used in the heart valve industry for years; more appropriate test models must be developed to be able to further develop living, tissue-engineered alternatives for introduction in the human clinical setting.

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Valvular Myofibroblast Activation by Transforming Growth Factor-β. Implications for Pathological Extracellular Matrix Remodeling in Heart Valve Disease

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The pathogenesis of cardiac valve disease correlates with the emergence of muscle-like fibroblasts (myofibroblasts). These cells display prominent stress fibers containing $_{\alpha}$-smooth muscle actin ($_{\alpha}$-SMA) and are believed to differentiate from valvular interstitial cells (VICs). However, the biological factors that initiate myofibroblast differentiation and activation in valves remain unidentified. We show that transforming growth factor-β1 (TGF-β1) mediates differentiation of VICs into active myofibroblasts in vitro in a dose-dependent manner, as determined by a significant increase in $_{\alpha}$-SMA and the dramatic augmentation of stress fiber formation and alignment. Additionally, TGF-β1 and increased mechanical stress function

synergistically to enhance contractility. In turn, contractile valve myofibroblasts exert tension on the extracellular matrix, resulting in a dramatic realignment of extracellular fibronectin fibrils. TGF-β1 also inhibits valve myofibroblast proliferation without enhancing apoptosis. Our results are consistent with activation of a highly contractile myofibroblast phenotype by TGF-β1 and are the first to connect valve myofibroblast contractility with pathological valve matrix remodeling. We suggest that the activation of contractile myofibroblasts by TGF-β1 may be a significant first step in promoting alterations to the valve matrix architecture that are evident in valvular heart disease.

Key words: valvular heart disease • valvular interstitial cells • myofibroblasts • TGF-β1 • $_{\alpha}$-smooth muscle actin • contractility